STUDIES ON BIOCHEMICAL GENETICS OF THE GREY MULLET *Mugil cephalus* LINNAEUS

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DEDICATED TO MY PARENTS

DECLARATION

I hereby declare that this thesis entitled **"STUDIES ON BIOCHEMICAL GENETICS OF THE GREY MULLET *Mugil cephalus* LINNAEUS"** has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

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CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON BIOCHEMICAL GENETICS OF THE GREY MULLET Mugil cephalus LINNAEUS"** is the bonafide record of the work carried out by **Mr. S. VIJAYAKUMAR** under my guidance and supervision and that no part thereof has been presented for the award of any other Degree.

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CONTENTS

PREFACE

The exploitation of fish resources has recently become a serious commercial venture throughout the world. All fish resources being of natural origin the reasons for fluctuations in their abundance can also be natural. However, a single factor that appears to have caused wide fluctuations in their catch statistics may be the effect of man's uncontrolled or unscientific exploitation of these fish resources contributed by species of commercial importance. The 1989 annual marine fish production of India reached about 2.2 million. The marine fish resources of India are mainly composed of pelagic and demersal types.

The fishes popularly known as mullets form about 0.41 percent of total production of marine fish landings and about 0.7 percent of pelagic fish production of India during 1989. Mullets are considered to be extremely important food fish throughout the world and which are cultivated or farmed in many countries including India. Though more than sixty species of mullets may exist, the largest and the most important species of commercial interest throughout the world is Mugil cephalus. Hence, the species has been subjected to very detailed biological investigation especially abroad. However, basic informations on the modern field of investigation, namely, the population genetics of M. cephalus are totally lacking, particularly in India. As a thorough knowledge of population genetics of M. cephalus of India is essential for its scientific exploitation at capture and culture levels, a doctoral research programme on the biochemical genetics of M. cephalus was undertaken. The important genetic informations found out for the first time are presented in the thesis. Considering the enormous potential genetic informations that may exist in the species, the findings made in the present investigation cannot be exhaustive. However, the biochemical genetic data collected, interpreted and critically evaluated are sufficient to generate an original conclusion on the biochemical genetic characteristics of three populations of M. cephalus tested in the present investigation. The important findings presented in the thesis will have definite academic and applied values.

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LIST OF ABBREVIATIONS USED

Tissues:

S / SER	=	Serum
M / MUS	=	Muscle
E / EYL	=	Eye lens
H / HAT	=	Heart
L / LIV	=	Liver
I / INS	=	Intestine
K / KID	=	Kidney

Buffer Systems:

BOR	=	Borate
TBO	=	Tris Borate
TCT	=	Tris Citrate
TCL	=	Tris Citric Boric Lithium OH
TGH	=	Tris Glycine HCl
TME	=	Tris Maleic EDTA
TVB	=	Tris Versene Borate
SCT	=	Sodium Citrate

Enzymes/Proteins:

ACP	=	Acid Phosphatase
ADH	=	Alcohol Dehydrogenase
ADO	=	Aldehyde Oxidase
ALD	=	Aldolase
AKP	=	Alkaline Phosphatase
EST	=	Esterase
GDH	=	Glutamate Dehydrogenase
GOT	=	Glutamate-Oxaloacetate Transaminase

GPD	=	Alpha-Glycerophosphate Dehydrogenase
LDH (E)	=	Lactate Dehydrogenase (Eye lens)
LDH (M)	=	Lactate Dehydrogenase (Muscle)
MDH	=	Malate Dehydrogenase
ODH	=	Octanol Dehydrogenase
PDH	=	Pyrroline Dehydrogenase
SDH	=	Sorbitol Dehydrogenase
TZO	=	Tetrazolium Oxidase
PRO (E)	=	Protein (Eye lens)
PRO (M)	=	Protein (Muscle)
PRO (S)	=	Protein (Serum)

Others:

COH	=	Cochin
MAD	=	Madras
ORI	=	Orissa
OBS	=	Observed
(Exp)	=	Expected
GRP	=	Group
FIG	=	Figure
TBL	=	Table
PLT	=	Plate
TOL	=	Total
D.D.W.	=	Double Distilled Water
(V/V)	=	Volume/Volume
(W/V)	=	Weight/Volume
B	=	Better
G	=	Good
P	=	Poor
(X)	=	Intensity
1X	=	Light Intensity
2X	=	Medium Intensity
3X	=	Dark Intensity
C	=	Colourless

1. INTRODUCTION

Fish resources of all kinds of water systems are an important source of cheap proteins from time immemorial. Though world fisheries are potentially self renewable resources, their unscientific exploitation can cause the component species that contribute to the fishery to reach the undesirable state of threatened species. Genetically speaking, such a level of exploitation of fish species also lead to unreplaceable loss of germ plasam resources. Therefore, a thorough knowledge of natural units or otherwise called genetic stocks of the fisheries under exploitation is essential. An advanced knowledge of such units of fish resources should help to build up a fishery management strategy in terms of scientific exploitation and genetic conservation of each natural unit of a fishery resource. Naturally, the question arises, what can be the unit of fishery resources to be managed. Is it the species itself or any other sub-units which may naturally exist within the species ?. The above question is more pertinent in the light of recent scientific practice of aquaculture of many species of fishes and shellfishes which has reached a state of modern industry throughout the world (Bye and Ponniah, 1983).

The species concept in natural history emerged during classical periods of Linnaeus, Lamark and Darwin. Darwin's theory of species evolution aparantly helped to explain wide' spread biological diversity distinctly observable within and between species and at higher levels of species organization. However, the enigmatic problems of mechanism of evolution could not be explained by Darwin's theory of evolution though he believed the inheritance of acquired characteristics. Meanwhile, Mendel's Law explained the basic mechanism of inheritance of observable particular individual variations. Though Darwin was also aware of the concept of geographic races within a species and their evolution into new species, the mathematical explanations were latter evolved by the dynamic theories of geographic speciation by his successors like Wagner, Jorden, Densch and Mayr (Mangaly, 1974). These theories explained how natural selection of variations can lead to differentiation of populations into races or subspecies (Dobzhansky, 1967, 1970).

The knowledge of the existence of geographical races or subspecies have great application in anthropology and biology primarily for the purpose of classification. The conventional concept of subspecies, races etc. is of fundamental importance in fisheries research and management. Because it helped in the demarcation of natural units of fisheries management. Thus the necessity of identification of natural units of economically important fish resources like herring and cod was evidently demonstrated by Heincke and Schmidt as early as 1898 and 1909 respectively. The basic reasons for identification of natural units of fish resources were summarised by Muzinic and Marr (1960) as "The logical and practical reasons for identifying population units are that such units may have their own characteristics of recruitment, growth, natural mortality, migration behaviour etc. more or less independent of the characteristics of other population units within the same species. The need for studies of population units in a particular situation may arise from two sources: (a) their exist variations in yield and/or heterogeneities in the characteristics of the catch for which the most likely explanation (hypothesis) is the existence of population units (b) management decisions have been made empirically for which it is necessary or desirable to provide understanding and thus confidence in the original decisions".

On the basis of above reasons of necessity of identification of fishery units the following questions also naturally arise regarding a definition of such natural units of fisheries management and its geographical range including its spawning range etc. (Marr, 1957; Marr and Sprague, 1963). A traditional unit was defined as "a relatively homogeneous and self contained population whose losses by emigrations and succession by emigrations if any, are negligible in relation to the rates of growth and mortality rate (Anon, 1960). The above traditional definition of fisheries management unit in terms of relative homogeneity is without any precise limiting scientific parameters. In modern times, fishery biologists and fishery management people prefer to define unit of fisheries resources in terms of Mendelian populations. A Mendelian population can be defined as "a reproductive community of sexual and cross-

fertilized individuals among whom matings regularly occur and who, consequently have a common gene pool" (Dobzhansky, 1967).

Though the purpose of the former conventional unit stock definition and that of modern population genetics is same, there are fundamental differences between the methods of collection of data required for realizing the defined unit stock structure of fisheries management. The former depends mainly on phenotypic variations, the sources and controlling factors of which are unknown whereas the latter wholly depends on genotypic variations, the source and controlling factors of which are known in advance (Moller, 1971; Jamieson, 1974a). The modern methods of collection of data in the form of visible and countable genotypic variation involves technique of gel electrophoresis and specific protein staining. The technique well established by Smithies (1955) for detection of human serum protein genotype variations was later modified by Hunter and Markert (1957) to discover enzyme genotype variations also. These techniques, popularly known as biochemical genetic techniques, are widely applied to obtain basic data required in delineating genetic unit stocks of numerous economically important fish resources exploited all over the world. These basic data exist in the form of numerous proteins in different tissues of individual fish composing the species (Utter, 1987).

The grey mullet Mugil cephalus (L.) is the largest and economically the most important mullet species of the family Mugilidae. It is widely distributed in Eastern Indian Ocean and Western Central Pacific. It being a euryhaline species, it occurs along coastal waters migrating into estuaries and rivers. It is also cultivated in different ecological conditions. It has been subjected to detailed biological investigations including controlled breeding programme in different parts of the world (Oren, 1981).

M. cephalus of Indian waters is not only economically important fish but also an esteemed table fish in Kerala. It is being exploited heavily in both west and east coast of India. Though it was caught abundantly about two decades back in Chinese dipnets of Cochin, it has become scarce in

the catches of these nets of Cochin in recent times. Though certain informations on its fishery and biology are known (Luther, 1967, 1973; Rangaswamy, 1972a, 1974, 1976, 1978; Jhingran, 1991) reports on the population characteristics, particularly, on the unit stock structure of M. cephalus from India are not available. Hence, it was planned to conduct an electrophoretic investigation of its different tissue proteins to reveal biochemical genetic characteristics of populations of Indian M. cephalus. The investigation was particularly planned to reveal the genetic stock structure of M. cephalus populations exploited from Cochin, Madras and Orissa regions. The present investigation of biochemical genetics of M. cephalus is also in line with the recommendations and world-wide programmes of United Nation Environment Programmes (UNEP), Food and Agricultural Organisation (FAO) and National Bureau of Fish Genetic Resources (ICAR) India, which are seriously concerned with the problems of conservation of genetic resources of animals and plants throughout the world (Jhingran, 1984; Das and Jhingran, 1989).

2. REVIEW OF LITERATURE

The pioneering work of Pauling et al. (1949) on sickle-cell haemoglobin types revealed the potential application of electrophoretic technique in discovering and visualizing the heterogeneity of similar proteins (Wilson and Kaplan, 1964). The discovery of electrophoretic variations of human serum proteins by Smithies (1955) and that of enzymes by Hunter and Markert (1957) enabled several investigators in biology to visualize variability of different types of proteins at different levels of species organization, including that of fishes. Sick (1961) reported haemoglobin polymorphism in fishes. The report of high degree of protein variability between natural populations of Drosophila pseudoobscura by Lewontin and Hubby (1966) and that of human by Harris (1966) encouraged researchers in various fields of biology to discover biochemical genetic variations in populations of different species including fishes. Meanwhile, the theory, methods and application of different electrophoretic techniques were also made available in the works of Smith (1960/1968), Ornstein and Davis (1964), Bier (1967) and Brewer (1970) and as a result of which large number of reports on genetic variability of protein appeared in the literature.

de Ligny (1969) reviewed "Serological and Biochemical studies on fish populations". The importance of informations on biochemical genetic data of fish stocks was well emphasised during the ICES Special Meeting held at Dublin (de Ligny, 1971). A general review of literature shows that inter and intraspecies variations of proteins are reported under two popular headings, namely, (1) general proteins and (2) enzymes or alternatively, non enzymatic and enzymatic proteins respectively. Major general proteins investigated for inter and intraspecies genetic variations are eye lens protein and muscle protein or myogen (Tsuyuki et al., 1965a, 1965b; Tsuyuki and Roberts, 1966; Barrett and Williams, 1967; Cowie, 1968; Tsuyuki et al., 1968; Eckroat and Wright, 1969; Mackei and Jones, 1969; Peterson and Shehadeh, 1971; Manohar and Velankar, 1973; Herzberg and Pasteur, 1975; Menezes, 1976a, 1976b; Rao and Dhulkhed, 1976; Bhosle, 1977; Jamieson and Turner,

1980; Smith et al., 1980; Winans, 1980; Bhattacharya and Alfred, 1982; Basiao and Taniguchi, 1984; Hines and Yashouv, 1970 Sbordoni et al., 1986; Mahobia, 1987; Philip Samuel, 1987; Fevolden and Haug, 1988; Salini and Shaklee, 1988; Chakraborty, 1990).

However, major portion of literature available for reference on protein variations in fishes are concerned with enzymatic proteins (isoenzymes). Because the potential number of enzyme systems that may be searched for genetic variations can be simply guessed from the fact that a single citric acid cycle biochemical process involves catalytic participation of a number of enzymes. Hence, enzyme systems have become favourite proteins for biochemical genetic investigations. Practical laboratory techniques to easily detect thirty eight enzyme systems were published by Shaw and Koen (1968) and Brewer (1970). These enabled to discover and report large volume of informations on electrophoretic characteristics of these enzymes in both invertebrate and vertebrate organisms including fishes. Recently enzyme techniques particularly suitable for specific group of aquatic animals like prawns were also made available (Shaklee and Salini, 1983). Shaw (1965) reviewed reports of electrophoretic variation in enzymes and their application as a research tool and their biological significance in several invertebrates and vertebrate organisms. Lush (1966) reviewed enzymatic and non-enzymatic variations in vertebrates. Latner and Skillen (1968) produced a monograph on the application of isoenzymes in biology and medicine (Mangaly, 1974). de Ligny (1972) discussed blood groups and biochemical polymorphism in fishes. Wilkins (1972) reviewed biochemical genetics of Atlantic salmon (Salmo salar).

Though Laboratory techniques for electrophoretic detection of thirty eight enzyme systems were given by Shaw and Koen (1968) and a rare report of electrophoretic analysis of thirty eight enzyme systems in the shark Carcharinus tilstoni and C. sorrah by Lavery and Shaklee (1989) are available, only, selected number of enzyme systems are investigated by majority of the authors. Krieg and Guyomard (1985) also analysed twenty three enzyme systems coded for fifty two loci in French brown trout Salmo trutta. However,

majority of investigators analysed lesser number of enzyme systems for studying biochemical genetic variation of fish populations.

An overall review of literature on electrophoretic analysis of enzymes in fishes, prawns and molluscs for inter and intraspecies genetic characterization shows that they come under three groups, namely (1) Hydrolases and lyases, (2) Dehydrogenases and (3) Dehydrogenase-coupled enzymes. The first group enzymes consist of Acid Phosphatase (ACP), Aldolase (ALD), Alkaline Phosphatase (AKP), Esterase (EST) and Leucine Aminopeptidase (LAP) etc. The second consists of mainly Alcohol Dehydrogenase (ADH), Alpha-Glycerophosphate Dehydrogenase (GPD), Glyceraldehyde-3-phosphate Dehydrogenase (G-3-PD), Glucose-6-phosphate Dehydrogenase (G-6-PD), Isocitrate Dehydrogenase (IDH), Lactate Dehydrogenase (LDH), Malate Dehydrogenase (MDH), Succinate Dehydrogenase (SCD) and Xanthine Dehydrogenase (XDH) etc. The third group consists of Adenylate Kinase (AK), Fumarase (FUM), Glutamate-Oxaloacetate Transaminase (GOT) or Aspartate amino transferase, Phosphoglucomutase (PGM) and Triose phosphate Isomerase (TPI) etc. (Smith, 1968).

The electrophoretic patterns of Acid phosphatase were studied in guppy (Shami and Beardmore, 1978), trout (Wishard et al., 1980), Cichlids (Kornfield and Koehn, 1975; Mahobia, 1987), sharks (Lavery and Shaklee, 1989), Ocean perch (Seeb and Gunderson, 1988), halibut (Fevolden and Haug, 1988), Crustaceans (Tracey et al., 1975b; Ayala and Valentine, 1977; Hedecock et al., 1977, 1979; Nelson and Hedgecock, 1980; Smith et al., 1980; De Matthaeis, et al., 1983; Sbordoni et al., 1986; Lavery and Staples, 1990). Alkaline phosphatase system was studied in mackerel (Smith and Jamieson, 1980), tilapia (Cruz et al., 1982), halibut (Fevolden and Haug, 1988) and Crustaceans (Takeo, 1970; Trudeau, 1978; Mulley and Latter, 1980; De Matthaeis et al., 1983; Sbordoni et al., 1986). Aldolase system was reported in fishes like yellow tail, flat fish, swell fish, sea bream and carp (Takeo, 1970), brown trout (Allendorf et al., 1976; Krieg and Guyomard, 1985), lake trout (Dehring et al., 1981) and Crustaceans (Selander et al., 1970; Nelson

and Hedgecock, 1980; Redfield et al., 1980; Hedgecock et al., 1982; De Matthaeis et al., 1983; Lester, 1983; Philip Samuel, 1987). In the first group of enzymes, Esterase enzyme system has been investigated in larger number of fishes like cat fish (Koehn and Rasmussen, 1967; Koehn, 1970), Pacific hake (Utter et al., 1970), zoarces (Christiansen and Frydenberg, 1974), American eel (Williams et al., 1973), sun fish (Avize and Smith, 1974), Cichlids (Kornfield and Koehn, 1975; Mahobia, 1987), European hake (Mangaly, 1974, 1978), guppy (Shami and Beardmore, 1978), New Zealand snapper (Smith et al., 1978; Smith, 1979), Atlantic mackerel (Smith and Jamieson, 1980; Smith et al., 1981a), milk fish (Winans, 1980), sprat (Smith and Robertson, 1981), New Zealand hoki (Smith et al., 1981b), tilapia (Cruz et al., 1982; McAndrew and Majumder, 1983; Basiao and Taniguchi, 1984), Australian barramundi (Shaklee and Salini, 1983; Salini and Shaklee, 1988), Pacific herring, Atlantic herring (Grant, 1984; Grant and Utter, 1984), perch (Gyllensten et al., 1985), Pacific cod (Grant et al., 1987), Northern pike (Seeb et al., 1987), brown trout (Krieg and Guyomard, 1985), shark (Lavery and Shaklee, 1989) and in Crustaceans (Johnson et al., 1974; Lester, 1979, Kannupandi, 1980; Mulley and Latter, 1980; De Matthaeis et al., 1983; Philip Samuel, 1987).

A review of recent literature on biochemical genetic investigation reveals that group II enzymes composed of different Dehydrogenases are being reported more than that of other two enzyme groups. The following literature reviewed below contains a majority of Dehydrogenases of group two enzymes and Dehydrogenases coupled group III enzymes. These enzymes were examined by the investigators mainly for the purpose of estimating allelic frequencies in different populations of the concerned species. Salmon and trout appeared to have been favourite fish species for examining allelic frequencies of the above mentioned two enzyme groups. As a result of thorough investigation on species of salmon a large volume of literature on their enzyme systems is available now (Aspinwall, 1973; Allendorf et al., 1976; Grant et al., 1980; Wishard et al., 1980; Dehring et al., 1981; Guyomard, 1981; Stoneking, 1981; Utter, 1981; Carl and Healy, 1984; Guyomard et al., 1984; Kreig and Guyomard, 1985; Thompson, 1985; Koljonen, 1986; Campton and Utter, 1987; Gharrett et al., 1987; Marnell et al., 1987; Quinn et al., 1987; Skaala and Jorstad,

1987; Wehrhahn and Powell, 1987; Berg and Gall, 1988; Verspoor 1988; Crozier and Moffett, 1990; Currens et al., 1990; Macaranas and Fujio, 1990; Hershberger, 1992). The other fishes investigated for these enzyme groups are American eel (Williams et al., 1973; Koehn and Williams, 1978), Cichlids (Scholl and Holzberg, 1972; Basasibwaki, 1975; Mahobia, 1987), atherinid (Johnson, 1975), mullets (Reddy et al., 1975; Rao, 1981; Rosenblatt and Waples, 1986; Campton and Mahmoudi, 1991), bait fish (Daly and Richardson, 1980), white fish (Imhof et al., 1980), New Zealand hoki (Smith et al., 1981b), tilapia (Cruz et al., 1982; Macaranas and Fujio, 1990), Jackass (Richardson, 1982a), sun fish (Avise and Smith, 1974; Avise et al., 1977) tilapia (Basiao and Taniguchi, 1984), herring (Grant, 1984a, 1984b, 1986), perch (Gyllensten et al., 1985; Seeb and Gunderson, 1988), perciforms tetradontiforms (Rosenblatt and Waples, 1986), cod (Grand et al., 1987), blenny (Presant, 1987), pike (Seeb et al., 1987), Atlantic halibut (Fevolden and Haug, 1988), shark (MacDonald, 1988; Lavery and Shaklee, 1989), baramundi (Salini and Shaklee, 1988), mosquitofish (Hernandez-Martich and Smith, 1990) and carps (Macaranas and Fujio, 1990; Sumantadinata and Taniguchi, 1990). These enzymes were also found in prawns (Mulley and Latter, 1980, 1981; Richardson, 1982b; Sbordoni, et al., 1986; Philip Samuel, 1987 Lavery and Staples, 1990) lobsters (Tracey et al., 1975b; Hedgecock et al., 1977), molluscs (Singh and Zouros, 1975a; Tracey et al., 1975a; Singh and Green, 1984).

The mode of inheritence and electrophoretic expression of some of these enzymes described above have been demonstrated by Shami and Beardmore (1978) in the fish guppy Poecilia reticulata. Recently literature on the interpretation of electrophoretic data from fisheries management point of view is also available (Allendorf et al., 1987; Allendorf and Ryman, 1987; Grant, 1987; Stahl, 1987; Utter et al., 1987). Many reports on fundamental and applied aspects of biochemical genetics mainly with reference to electrophoretic data on protein variations in fish populations and their significance in fisheries and aquaculture research and management programmes were published in special issue of Canadian Journal of Fisheries and Aquatic Sciences, Vol.38,

No.12, December 1981. Besides, several text books dealing with principles of General Genetics (Dobzhansky, 1967, 1970), Modern Genetics (Franswroth, 1978; Ayala and Kiger, 1980) and special publications like Application of Genetics in Aquaculture by Bye and Ponniah (1983) are also available for reference. Very recently, an awareness on the importance of fish genetic resources and its conservation and management in India, applying modern principles of genetic stocks concept, was generated in the publications of Jhingran (1984), Das and Jhingran (1989), and Das et al. (1989).

The published reports on biochemical genetics of Indian fishes are only a few of preliminary in nature. These reports are mainly attempts to find out interspecies or species specific protein differences in fishes like flat fish (Kasinathan et al., 1972) in marine fishes (Manohar and Velankar, 1973) in goboids (Natarajan et al., 1975) myogen patterns in Bombay duck (Kurian, 1977) proteins variations in oil sardine populations (Rao and Dhulked, 1976) in mackerel (Dhulked and Rao, 1976) in M. cephalus (Bhosle, 1977) protein and enzyme variations in mullets (Rao, 1981) in prawns (Thomas, 1981) in oyster (Ponniah, 1988) and genetic variations of enzymes in mackerel (Menezes, 1986; Menezes et al., 1990). Some important doctoral theses submitted on biochemical genetics of fishes and prawns are also available (Reddy, 1977; Rao, 1981; Mahobia, 1987; Philip Samuel, 1987).

3. MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 POPULATION SAMPLES

The population samples of M. cephalus were collected from three distant regions, Cochin (West Coast), Madras and Orissa (East Coast) (FIG.1; PLT.1,2). Due to scarcity of sample specimens, Cochin population sample originated from three different locations namely, Kumbalangi, Puduvyppu, and Vypin. The Madras population sample was collected mainly from Pulicate Lake, the rest from Ennore and Muttukkadu. The Orissa population sample was collected exclusively from Chilka Lake. For the purpose of differentiation of M. cephalus species from that of locally available common species of mullets, specimens of Liza parsia and Valamugil cunnesius were collected from Cochin (PLT.1,2). For detection of ontogenic variations specimens of M. cephalus belonging to a size range of 2.6 to 32.5 cms were also collected. From each specimen, nearly seven tissues, namely, Serum (S), Muscle (M), Eye lens (E), Heart (H), Liver (L), Intestine (I) and Kidney (K) were taken for studies. Heart, Liver and Kidney were washed thoroughly with ice-cold Double Distilled Water (D.D.W.) and dried those tissues with blotting paper so as to render them free from any blood tinge. Intestine was washed thoroughly to make sure that it was free from the intestinal contents. Sometimes whole specimens were also collected.

3.1.2 SAMPLE TRANSPORTATION

Fish sample from Cochin region was brought to the laboratory in iced condition. Samples from Madras and Orissa were brought to the laboratory in the form of tissues packed in the aluminium foil and put into the plastic vials. At the collection centre these vials were stored in an ice box containing ice. The ice box was immediately taken to the local nearest deep freezer facilities and the whole ice box with some added quantity of water was kept open in the deep freezer until a block of ice was formed within the ice box. Then the ice box containing the sample was immediately

FIG. 1

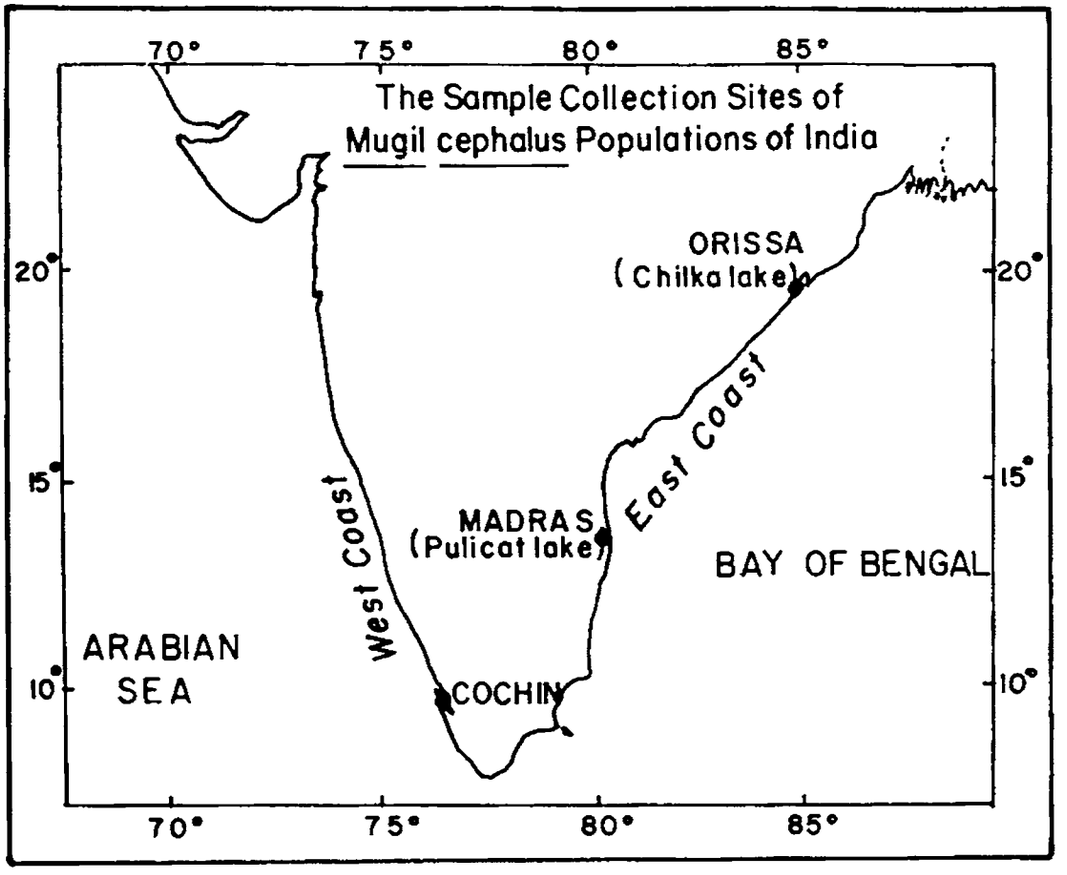
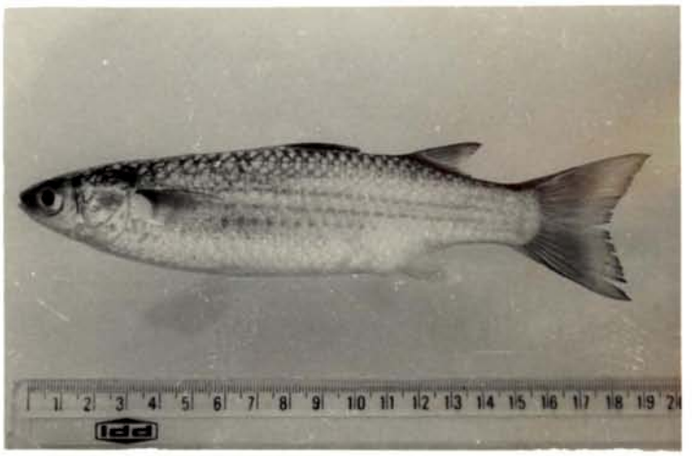
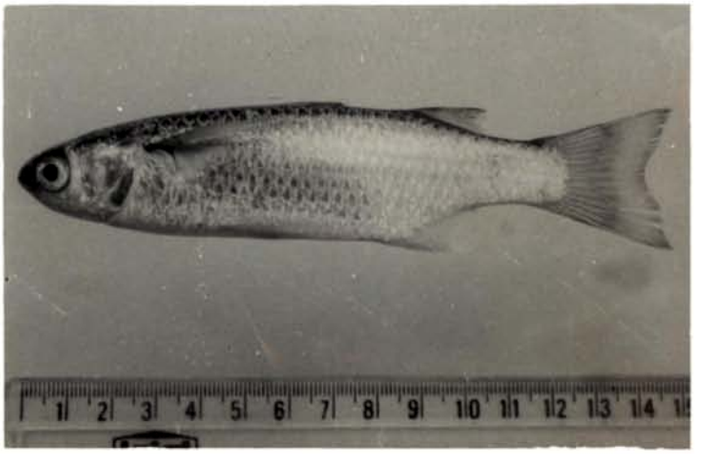

70°
75°
80°
85°
The Sample Collection Sites of
Mugil cephalus Populations of India
ORISSA
(Chilka lake)
20°
15°
10°
West Coast
East Coast
MADRAS
(Pulicat lake)
BAY OF BENGAL
ARABIAN
SEA
COCHIN

PLATE 1

Mugil cephalus



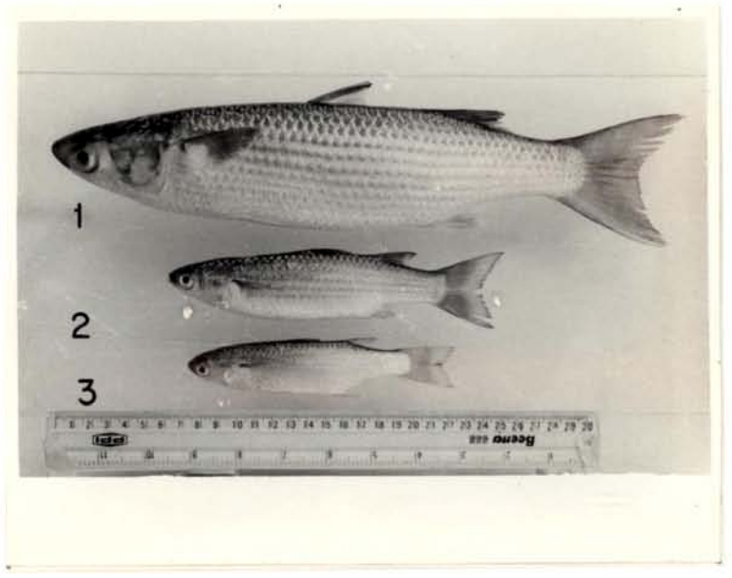
Liza parsia



Valamugil cunnesius

PLATE 2

THREE MULLET SPECIES FROM COCHIN



1. M. cephalus
2. L. parsia
3. V. cunnesius

transported to Cochin laboratory where the samples were immediately stored in the deep freezer until used for the experiment.

3.2 METHODS

3.2.1 SAMPLE EXTRACTION

All proteins/enzymes extraction were done in D.D.W. The tissues were homogenised with Remi Mechanical Homogeniser and centrifuged in Sorvall Refrigerated Superspeed Centrifuge. The supernatants obtained were used for electrophoretic analysis.

3.2.2. POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE)

The principle of electrophoresis is that charged ions or group will migrate towards one of the electrodes when placed in an electric field. The rate of electrophoretic migration will mainly depend on net charge differences, pH, though the shape and size of the molecules also influence the rate. So the rate of migration varies for different ions and this difference is used to separate the components of a protein mixture. The mixture of substance to be separated is placed as a narrow zone at a suitable distance from a particular electrode. As migration occurs, different protein components move at different rates towards the electrode of opposite charge. As most proteins are negatively charged, the protein mixture samples are applied at cathode region which then migrate towards the opposite positively charged anode region. This electrophoretic technique has been widely used in the separation of proteins, nucleic acids etc. The method followed here is that of Dietz et al. (1972).

3.2.3 REAGENTS FOR DISC GEL ELECTROPHORESIS

The following reagents were prepared as follows: The reagents were stored in refrigerator at 4°C until used.

Gel Buffer (Tris HCl, pH 8.9):

Tris Buffer (Tri-hydrozy methyl aminomethane) = 36.6 gm

1 N HCl	=	48.0 ml
TEMED (N, N, N', N' - Tetramethyl ethyline diamine)	=	0.26 ml

All the reagents were dissolved in Double Distilled Water (D.D.W.) and made upto 100 ml. The pH of the solution was checked with Toshniwal battery operated pH meter. The pH was adjusted by adding the concentrated HCl as required. The solution was filtered with Whatman paper and kept in an amber coloured bottle.

Electrode Buffer (Tris Glycine, pH 8.3):

Tris Buffer (0.05 M)	=	6 gm
Glycine (0.38 M)	=	28.8 gm

All the reagents were dissolved in D.D.W. (W/V), made upto one litre. Before use one part of this stock solution was diluted with nine (9) parts of water (V/V). So that the required molarity was obtained and the solution was used as electrode buffer (tank buffer).

Acrylamide (30%) and Bisacrylamide (0.8%):

30 gm of acrylamide (Monomer) and 0.8 gm of methylene-bis-acrylamide (dimer) were dissolved in 100 ml of D.D.W. and filtered (W/V). The solution was stored in an amber coloured bottle.

Ammonium per sulphate (0.0061 M):

140 mg of ammonium persulphate was dissolved in 100 ml of D.D. distilled water (W/V).

40% Sucrose:

40 gm of sucrose was dissolved in 100 ml of D.D.W. and filtered.

Trace Solution:

100 gm of bromophenol blue was dissolved in 100 ml of D.D.W. and filtered.

3.2.4 PROCEDURE FOR GENERAL PROTEIN ELECTROPHORESIS

Preparation of 7.5% Gel:

The stock solutions, like, Gel Buffer, Acrylamide/Bisacrylamide and Ammonium persulphate were brought under room temperature before preparation of the Gel. To obtain 7.5% gel, 3.5 ml of Gel Buffer, 7 ml of Acrylamide/ Bisacrylamide, 3.5 ml of D.D.W. and 14 ml of Ammonium per sulphate were thoroughly mixed. Care was taken to avoid trapping air which otherwise slowly rise as bubbles in the gel solution. Glass gel tubes of 100 mm length and 6 mm inner diameter were carefully inserted into the rubber grommet. The mixed solution was carefully added with help of a glass van syringe to a height of 80 mm. Immediately water was layered carefully above the gel top by a plastic syringe without disturbing the gel bed to a height of about 2-3 mm. The water layer over the gel solution helps to obtain a gel with a flat surface. The gel is formed in about 20-30 minutes at room temperature. After polymerisation the water was decanted from the gel top.

Sample Application and Electrophoresis:

A sample volume of 100 ul was applied by means of Finn-Micropipette over the gel. The sucrose solution containing 40 ul was also added along with sample to ensure a uniform flow of current as well as for holding the sample without diffusion into the tank buffer. It also helps in faster migration of the sample into the gel. The gel tubes with the samples were then inserted into the silicon rubber grommets of the upper buffer tank reservoir in such a way that the upper end of tubes holding the sample were just above the lower surface of the upper tank. the upper and lower buffer tanks were filled with the electrophoretic tank buffer. A few drops of bromophenol blue solution (fast moving dye) was added to the upper buffer tank solution to follow the migration level of the sample in the gel tube. The upper tank was fixed to the lower tank of the electrophoretic apparatus and the apparatus was connected to the power pack.

The power pack was switched on for 30 minutes before the apparatus was connected to it. So that the power pack got warmed and stabilized to help the current flow uniformly. The current was regulated to have an uniform flow of 12 milli Ampere (mA) for 10 minutes, then adjusted to 36 mA for twelve gel tubes. The power was switched off when the marker dye had migrated to about 5 mm from the lower end of the gel. The electrophoretic run was carried out at 4°C in a refrigerator. The electrophoresis usually took about 1½ hours. The gel tubes were removed from the grommets. Then the total length of the gel and distance travelled by the marker dye were measured. The gels were then removed from the gel tubes by forcing a jet of water between the gel and the inner wall of the gel tubes using a syringe without damaging the gels.

Staining Procedure for Protein:

1. The protein present in the gels were fixed by placing the gels in 10% trichloroacetic acid (TCA) solution for 10 minutes.
2. The gels were then stained in amidoblack (0.1% in 7% acetic and filtered before use) for 10 minutes.
3. Destaining was carried out by soaking the gels in 7% acetic acid until stains thoroughly washed off from the gel.

3.2.5 PROCEDURE FOR ENZYME ELECTROPHORESIS

Procedure for preparation of the gel for enzyme experiments was almost similar to that described above for protein.

Acrylamide Stock Solution (40%):

40 gm of acrylamide was dissolved in 100 ml of D.D.W. and filtered. The solution was kept at 4°C in an amber coloured bottle (W/V).

Bis Acrylamide (2.1%):

2.1 gm of bis acrylamide was dissolved in 100 ml of D.D.W. and filtered (W/V).

Buffer Systems:

A variety of buffer systems given in literature were tried for finding out suitable buffers that gave better electrophoretic separation and resolution of proteins/enzymes tested here. The details of the buffer systems tried in the present experiments are given in Table 2.

Gel Composition:

Based on the Table 1 different types of gel were prepared using different gel compositions. These differences helped to produce different pore sized gels. For enzyme separation the gel tubes were smaller in size than that of protein. Smaller gels helped to reduce the consumption of expensive staining solution.

Staining Procedure for Enzyme:

After the electrophoresis the gels were incubated in a staining solution specific for a particular enzyme. The staining solution containing substrate for each enzyme, co-factor NAD, electron acceptor PMS, electron indicator NBT and one buffer to maintain pH were added. Staining solutions required for detection of specific enzymes were prepared as shown below:

1. **Acid Phosphatase: (Shaw and Prasad, 1970):**

Stain Buffer	=	100 ml
Sodium alpha napthyl acid phosphate	=	100 mg
Fast Garnet GBC salt	=	100 mg

The gel immersed in the above solution was incubated at 37°C until Red bands appeared.

Stain buffer:

Na acetate 3 H_2O	=	6.8 gm
1 N HCl	=	14.8 ml

Made up to 1 litre in D.D.W. and
adjusted the pH to 5.0 with 1 N HCl.

TABLE 1

Details of Gel Compositions

Acrylamide 40 gm / 100 ml.	Bisacrylamide 2.1gm / 100 ml.	D.D.W.	Gel Buffer	Ammonium Persulphate 140 mg / 100 ml.	Total Volume
5.0 ml	5 ml	--	5 ml	5 ml	
5.0 ml	4 ml	1.0 ml	5 ml	5 ml	
5.0 ml	3 ml	2.0 ml	5 ml	5 ml	
5.0 ml	2 ml	3.0 ml	5 ml	5 ml	
4.5 ml	5 ml	0.5 ml	5 ml	5 ml	
4.5 ml	4 ml	1.5 ml	5 ml	5 ml	
4.5 ml	3 ml	2.5 ml	5 ml	5 ml	
4.5 ml	2 ml	3.5 ml	5 ml	5 ml	
					20 ml
3.5 ml	5 ml	1.5 ml	5 ml	5 ml	
3.5 ml	4 ml	2.5 ml	5 ml	5 ml	
3.5 ml	3 ml	3.5 ml	5 ml	5 ml	
3.5 ml	2 ml	4.5 ml	5 ml	5 ml	
2.5 ml	5 ml	2.5 ml	5 ml	5 ml	
2.5 ml	4 ml	3.5 ml	5 ml	5 ml	
2.5 ml	3 ml	4.5 ml	5 ml	5 ml	
2.5 ml	2 ml	5.5 ml	5 ml	5 ml	

TABLE 2 Electrophoresis Buffer systems

Sl. No.	Buffers	Electrode Components (per liter)	pH	Gel Components (per liter)	pH	Nature of Buffer Systems	Reference
I	Borate (0.341M) (BOR)	Boric acid (0.341M) = 21.10 gm NaOH = 5.32 gm	9.0	Boric acid = 2.11gm Na OH = 0.60gm	9.0	Continuous	Shaw and Prasad (1970)
II	Tris Borate (0.0546M Tris 0.2454M Borate) (TBO)	Tris (0.0546M) = 6.61 gm Boric acid (0.2454M) = 15.17 gm	7.5	Tris = 0.12gm Boric acid = 1.79gm	7.5	Continuous	Shaw and Prasad (1970)
III	Tris Citrate (0.155M Tris 0.043M Citric acid (TCT)	Tris = 16.35 gm Citric acid = 9.04 gm	7.1	Dilute 6.67 ml of electrode buffer to 1 liter	7.1	Continuous	Shaw and Prasad (1970)
IV	Tris Citric Boric Lithium OH (TCL)	Li OH = 2.51 gm Boric acid = 18.54 gm	8.26	Tris = 3.63gm Citric acid = 1.05gm Electrode Buffer = 10 ml	8.31	Discontinuous	Ferguson and Wallace (1961)
V	Tris Glycine HCl (TGH)	Tris = 6.00 gm Glycine = 28.80 gm	8.3	Tris = 36.60gm TEMED = 0.26ml IN HCl = 48 ml	8.9	Discontinuous	Davis (1964)
VI	Tris Maleic EDTA (0.1M Tris 0.1M Maleic 0.01M EDTA (TME)	Tris = 12.10 gm Maleic = 11.60 gm $Na_2EDTA.2H_2O$ = 3.72 gm $MgCl_2.6H_2O$ = 2.03 gm	7.6	Dilute electrode Buffer 1:10 times with H_2O	7.6	Continuous	Shaw and Prasad (1970)
VII	Tris Versene Borate (0.5M Tris) (TVB)	Tris = 60.60 gm Boric acid = 40.00 gm $Na_2EDTA.2H_2O$ = 6.00 gm	8.0	Tris = 6.06gm Boric acid = 6.00gm Na_2 EDTA = 0.60gm	8.0	Continuous	Shaw and Prasad (1970)
VIII	Sodium Citrate (SCT)	Sodium Citrate = 120.58 gm pH is adjusted with 0.41M Citric acid	7.0	Histidine = 0.78gm pH is adjusted with 2N Na OH	7.0	Discontinuous	Brewer (1970)

2. Alcohol Dehydrogenase: (Siciliano and Shaw, 1976):

NAD	=	25 mg
MTT	=	15 mg
95% Ethanol	=	2 ml
Tris HCl (0.2 M) pH 8.5	=	7 ml
H_2O	=	41 ml

The gel was kept in the stain thus prepared and it was incubated at 37°C in dark. When dark blue bands appeared gel was rinsed in D.D. Water and fixed in 50 ml of Alcohol Gel wash. Alcohol wash was prepared as follows:

Alcohol Gel Wash:

Ethanol	=	1000 ml
Acetic acid	=	400 ml
Glycerine	=	200 ml
H_2O	=	800 ml

Tris HCl Buffer (0.2 M) pH 8.5:

24.2 gm Tris Buffer + 900 ml of H_2O made up to 1 litre in H_2O adjusted the pH to 8.5 with concentrated HCl.

3. Aldehyde Oxidase: (Redfield and Salini, 1980):

NAD	=	20 mg
MTT	=	10 mg
Benzaldehyde	=	1 ml
Tris HCl Buffer pH 8.5	=	50 ml
H_2O	=	50 ml
PMS	=	2 mg

Dark blue bands appeared when the gel was placed in the above stain solution.

4. Aldolase: (Siciliano and Shaw, 1976):

Fructose 1-6 diphosphate (Tetrasodium salt)	=	275 mg
NAD	=	25 mg
NBT	=	15 mg
Sodium arsenate	=	75 mg
Tris HCl Buffer (0.2 M) pH 8.0	=	10 ml
H_2O	=	40 ml
PMS	=	1 mg
Glyceraldehyde-3-Phosphate Dehydrogenase	=	100 units

When the gel was kept in the above staining solution at 37°C, dark blue band appeared.

Tris HCl Buffer (0.2 M) pH 8.0:

Dissolved 24.2 gm of Tris in 1000 ml of D.D. Water then adjusted the pH to 8.0 with concentrated HCl.

5. Alkaline Phosphatase: (Shaw and Prasad, 1970):

Beta-Napthyl sodium phosphate	=	50 mg
Fast Garnet GBC salt	=	50 mg
Mg SO_4 7 H_2O	=	123 mg
Tris HCl Buffer pH 8.5	=	100 ml

The gel in the staining solution was incubated at 37°C until red band appeared.

6. Esterase: (Redfield and Salini, 1980):

Fast Garnet GBC salt	=	100 mg
Alpha-Napthyl acetate solution	=	1.5 ml
Tris HCl Buffer pH 7.5	=	50 ml
H_2O	=	50 ml

Esterase activity was indicated by dark violet blue bands after incubating the gel in the stain at 37°C.

Napthyl acetate solution:

1 gm of Alpha-Napthyl acetate was dissolved in 50 ml of acetone, then 50 ml of H_2O added.

7. Glutamate Dehydrogenase: (Shaw and Prasad, 1970):

NAD	=	60 mg
NBT	=	30 mg
Phosphate Buffer (0.1 M) pH 7.0	=	25 ml
Na Glutamate	=	5 ml
H_2O	=	70 ml

Incubated the gel at 37°C in the dark, until dark blue bands appeared.

Phosphate Buffer (0.1 M) pH 7.0:

Pottasium phosphate (K_2HPO_4)	=	17.42 gm
Pottasium dihydrogen orthophosphate (KH_2PO_4)	=	13.61 gm
H_2O	=	1000 ml

Na Glutamate:

Dissolved 16.9 gm of L-Glutamic acid in 250 ml of 0.1 M phosphate Buffer.

8. Glutamate-Oxaloacetate Transaminase: (Shaw and Prasad, 1970):

L. Aspartic acid	=	532 mg
Alpha-Ketoglutaric acid	=	73 mg
Pyridoxal phosphate	=	50 mg
East Garnet GBC salt	=	200 mg
0.1 M phosphate Buffer pH 7.0	=	100 ml

Incubated at 37°C until bands appeared. Fixed the gel in glycerine (Bands diffuse in the usual fixing solution).

9. Alpha-Glycerophosphate Dehydrogenase: (Siciliano and Shaw, 1976):

Sodium Alpha-Glycerophosphate	=	75 mg
NAD	=	25 mg
NBT	=	15 mg
Tris HCl Buffer (0.2 M) pH 8.0	=	10 ml
H_2O	=	40 ml

Gels treated with above solution at 37°C produced dark blue bands of enzyme activity.

10. Lactate Dehydrogenase: (Davis, 1964):

NAD	=	7.2 ml
Lithium lactate	=	12.0 ml
NBT	=	24.0 ml
H_2O	=	28.8 ml
PMS	=	2 mg

Incubated the gel at 37°C in the dark, until blue bands appeared. Washed in 7% acetic acid and fix in D.D. Water.

LDH Staining Buffer:

Tris HCl Buffer (0.2 M) pH 8.5:

Dissolved 24.2 gm of Tris in 1000 ml of D.D. Water, then adjusted the pH to 8.5 with concentrated HCl.

Stock solution used for staining:

NAD: (10 mg/ml):

100 mg of NAD dissolved in 10 ml of staining buffer.

Lithium Lactate: (30 mg/ml):

750 mg of Lithium lacate dissolved in 25 ml of staining buffer.

NBT (0.9 mg/ml):

45 mg of NBT dissolved in 50 ml of staining buffer.

11. Malate Dehydrogenase: (Siciliano and Shaw, 1976):

Malic acid	=	750 mg
Na_2CO_3	=	1.25 gm
NAD	=	25 mg
NBT	=	15 mg
Tris HCl Buffer (0.2 M) pH 8.5	=	15 ml
H_2O	=	35 ml

Incubated the gel in the above staining solution in dark at 37°C. Zones of enzyme activity appeared as dark blue bands.

12. Octanol Dehydrogenase: (Redfield and Salini, 1980):

Octanol	=	3 ml
NAD	=	20 mg
MTT	=	10 mg
Tris HCl Buffer (0.2 M) pH 8.5	=	50 ml
H_2O	=	47 ml
PMS	=	2 mg

The gel immersed in the above staining solution was incubated at 37°C to get bluish pink coloured bands.

13. Pyrroline Dehydrogenase: (Redfield and Salini, 1980):

Alpha-Pyroglutamic acid	=	50 mg
NAD	=	20 mg
MTT	=	10 mg

Tris HCl Buffer (0.2 M) pH 8.0	=	50 ml
H_2O	=	50 ml
PMS	=	2 mg

Incubate gel at 37°C until blue bands appear wash and fix.

14. Sorbitol Dehydrogenase: (Redfield and Salini, 1980)

D - Sorbitol	=	1 gm
NAD	=	20 mg
MTT	=	10 mg
Tris HCl Buffer (0.2 M) pH 8.0	=	50 ml
H_2O	=	50 ml
PMS	=	2 mg

Incubated the gel in the above staining at 37°C to gel blue colour bands.

3.2.6 STANDARDIZATION OF EXPERIMENTAL CONDITIONS

To obtain good resolution and separation of the bands of each enzyme nearly four different buffers were tested for best results. Similarly to find out the best tissue source for each enzyme, seven different tissue samples were tested and compared. The optimum quantity of the samples to be extracted (mg/ml) and the extracts to be loaded (µl) in a gel also were standardized. Table 3 shows the final experimental details for the screening test.

3.2.7 LIST OF ENZYMES/GENERAL PROTEINS TESTED

1.	Acid Phosphatase	(ACP)
2.	Alcohol Dehydrogenase	(ADH)
3.	Aldehyde Oxidase	(ADO)
4.	Aldolase	(ALD)
5.	Alkaline Phosphatase	(AKP)
6.	Esterase	(EST)

7.	Glutamate Dehydrogenase	(GDH)
8.	Glutamate-Oxaloacetate Transaminase	(GOT)
9.	Alpha-Glycerophosphate Dehydrogenase	(GPD)
10.	Lactate Dehydrogenase	(LDH)
11.	Malate Dehydrogenase	(MDH)
12.	Octanol Dehydrogenase	(ODH)
13.	Protein	(PRO)
14.	Pyrroline Dehydrogenase	(PDH)
15.	Sorbitol Dehydrogenase	(SDH)
16.	Tetrazolium Oxidase	(TZO)

3.2.8 PROCEDURES FOR ANALYSIS OF DATA

1. Interpretation of Electrophoretic Patterns (FIG. 72):

The protein/enzyme banding patterns or the phenotypes were compared between individuals at a particular gel area. Phenotype variants observed between the individuals in terms of differences in the distance travelled by particular band **(S)** at that particular compared gel area are designated as slow moving **(S)** in one individual, fast moving **(F)** in another individual and slow-fast moving **(SF)** when a combination of these two occured yet in another individual. As a standard practice, these protein phenotypes are presumed as genotypes produced by codominant alleles at a particular genetic locus. Thus individuals having **S, F, SF** genotypes were scored as slow moving and fast moving homozygotes and slow-fast moving heterozygote respectively. Depending on the protein/enzyme structure, the observed heterozygote patterns are again scored as monomeric, dimeric or tetrameric, viz. two, three and five banded patterns respectively.

2. Allelic Frequencies (TBL. 21):

Allelic frequencies were calculated directly from genotype frequencies. Genotype frequencies are proportions of each genotype in total number of individuals tested for each locus.

TABLE 3 *

Final Experimental Details for Screening Test.

Sl. No.	Enzymes	Buffers	Gel Concentrations Acrylamide ml / Bisacrylamide ml.	Tissues	Sample Concentration
1	ACP	BOR	2.5 ml / 2 ml	LIV	100 mg / ml
2	ADH	TGH	3.5 ml / 2 ml	LIV	100 mg / ml
3	ADO	TGH	3.5 ml / 2 ml	LIV	100 mg / ml
4	ALD	TVB	3.5 ml / 2 ml	LIV	100 mg / ml
5	AKP	BOR	3.5 ml / 2 ml	LIV	100 mg / ml
6	EST	TVB	5.0 ml / 3 ml	HAT	40 mg / ml
7	GDH	TCT	3.5 ml / 2 ml	EYL	40 mg / ml
8	GOT	SCT	3.5 ml / 2 ml	MUS	100 mg / ml
9	GPD	TVB	2.5 ml / 2 ml	LIV	100 mg / ml
10	LDH (E)	TGH	4.5 ml / 3 ml	EYL	40 mg / ml
11	LDH (M)	TGH	3.5 ml / 2 ml	MUS	100 mg / ml
12	MDH	TCT	2.5 ml / 2 ml	LIV	100 mg / ml
13	ODH	TGH	3.5 ml / 2 ml	LIV	100 mg / ml
14	PDH	TVB	3.5 ml / 2 ml	EYL	40 mg / ml
15	SDH	TCL	3.5 ml / 2 ml	LIV	100 mg / ml
16	TZO	TVB	3.5 ml / 2 ml	LIV	100 mg / ml
17	PRO (E)	TGH	7.5 %	EYL	40 mg / ml
18	PRO (M)	TGH	7.5 %	MUS	100 mg / ml
19	PRO (S)	TGH	7.5 %	SER	100 µl / 900 µl

* Refer Page xvi for explanation of abbreviations

Thus frequency of **S** allele	=	Frequency of **SS** genotype plus half the frequency of **SF** genotype
Frequency of **F** allele	=	Frequency of **FF** genotype plus half the frequency of **SF** genotype

Allelic frequency can also be calculated using the formula:

$$\frac{HO \times 2 + He}{2N}$$

Where HO is the observed number of a particular homozygote, **He** is observed **the** number of a particular heterozygote and **N** is the total number of individuals tested.

3. Expected Genotype Frequencies (TBL. 23):

Expected genotype frequencies, as per Hardy-Weinberg Law, were **calculated** using the binomial expansion

$$(a = b)^2 = a^2 + (2\ ab) + b^2$$

When two variant alleles were involved and multinomial expansion $(a + b + c.....)^2$ when more than two variant alleles were considered.

Chi-Square values for determining the significance of deviation between **observed** and expected genotype frequencies were calculated using the formula.

$$\frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}}$$

The degrees of freedom for determining the P value were calculated **from** the number of phenotypes minus 1, minus the number of alleles minus 1 (Utter, 1987). Thus the degrees of freedom to be considered in diallelic **three** phenotype system is

3 Phenotypes - 1 = 2

2 Alleles - 1 = 1

The degrees of freedom = 2-1 = 1

4. Heterozygosity (TBL. 24):

Heterozygosity was directly estimated from the number of heterozygotes present in the total number of individuals tested. Average heterozygosity in the species was calculated by estimating heterozygosity for each locus in each population, followed by their averages for the total loci for each population, followed by the average for the number of populations tested. Both polymorphic and non-polymorphic loci tested were considered for calculation.

5. Average number of alleles per locus (TBL. 22):

First, average number of alleles for each protein locus in each population was calculated. From these average number of alleles for different loci, average value for all loci for each population was calculated. From these population averages, average for the species was calculated.

6. Polymorphic loci (TBL. 20):

A locus was considered polymorphic when the most common allele had a frequency of 0.95 or less. The frequency of polymorphic loci was calculated as follows. First, proportions of loci polymorphic in the total number of loci present in each protein/enzyme tested were calculated for each population. Thus averages for all the proteins/enzymes tested for each population were calculated. From these values average for each population was calculated. The average for the species was calculated from averages of different populations.

7. Genetic Identity (I) and Genetic Distance (D) (TBL. 26):

The method followed is as described by Utter (1987).

Nei's (1975) genetic distance is defined as

D = -1 n (I) for a single locus

Genetic identity (I) is calculated using the formula

$$1 = \sum X_i Y_i \Big/ \sqrt{\sum X_i^2 \quad Y_i^2}$$

Where value of X_i and Y_i are the frequencies of specific alleles in the population X and Y, respectively. When more than one locus is involved in a protein system tested, average of (I) value for all loci considered is taken for calculating the (D) value for that protein system between X and Y populations. Then average (I) and (D) values for all loci tested for two populations thus compared are found out. From these average (I) and (D) values of compared populations, average for the species was estimated.

4. RESULTS

4.1 STANDARDIZATION OF EXPERIMENTS

The purpose of standardization of disc gel electrophoretic techniques applied in the present investigation was maximum separation and resolution of different components of specific proteins/enzymes tested. To achieve the above objective, several buffer systems, different gel percentage compositions, different tissue extracting processes, different proportions of sample volume, different staining processes etc. were experimentally compared and evaluated the results. The experimental details of general proteins and fourteen enzymes systems are shown in Figure 2-57, Plate 3-13 and Table 1-17. A comparison of these results show that a thorough standardization procedure involving trial and error method alone can produce presence of all the protein systems and their components in a particular species like M. cephalus. Because tissues that did not show any or poor enzyme activity in a particular procedure produced excellent patterns when a different detection method was applied. For selection of a suitable buffer system a group of four selected buffer systems already known suitable for certain other species were tried during the standardization of fourteen enzyme systems studied in the present investigation. However, in the case of general proteins, as the Tris-Glycine-HCl buffer system (TGH) gave good resolution of proteins, no other buffer systems were used for standardization. The important aspects of results of standardization experiments conducted in the present investigation can be described as follows. The Table 2 and 3 show that Borate Buffer system (BOR) was most suitable for Acid Phosphatase and Alkaline Phosphatase enzymes whereas Tris-Glycine-HCl buffer system (TGH) was better for ADH, ADO, LDH and ODH enzymes. Similarly, Tris-Versene-Borate buffer system (TVB) produced better results in the case of ALD, EST, GPD and PDH enzymes. Tris-Citrate system (TCT) produced better results for GDH and MDH. Tris-Citric-Boric-LiOH (TCL) buffer system produced satisfactory patterns for SDH enzyme. Table 1 and 3 also show different proportions of Acrylamide and Bisacrylamide proportions required for better separation of each enzyme. These proportions were taken from respective stock solutions. Thus Acrylamide - Bisacrylamide

FIG. 2

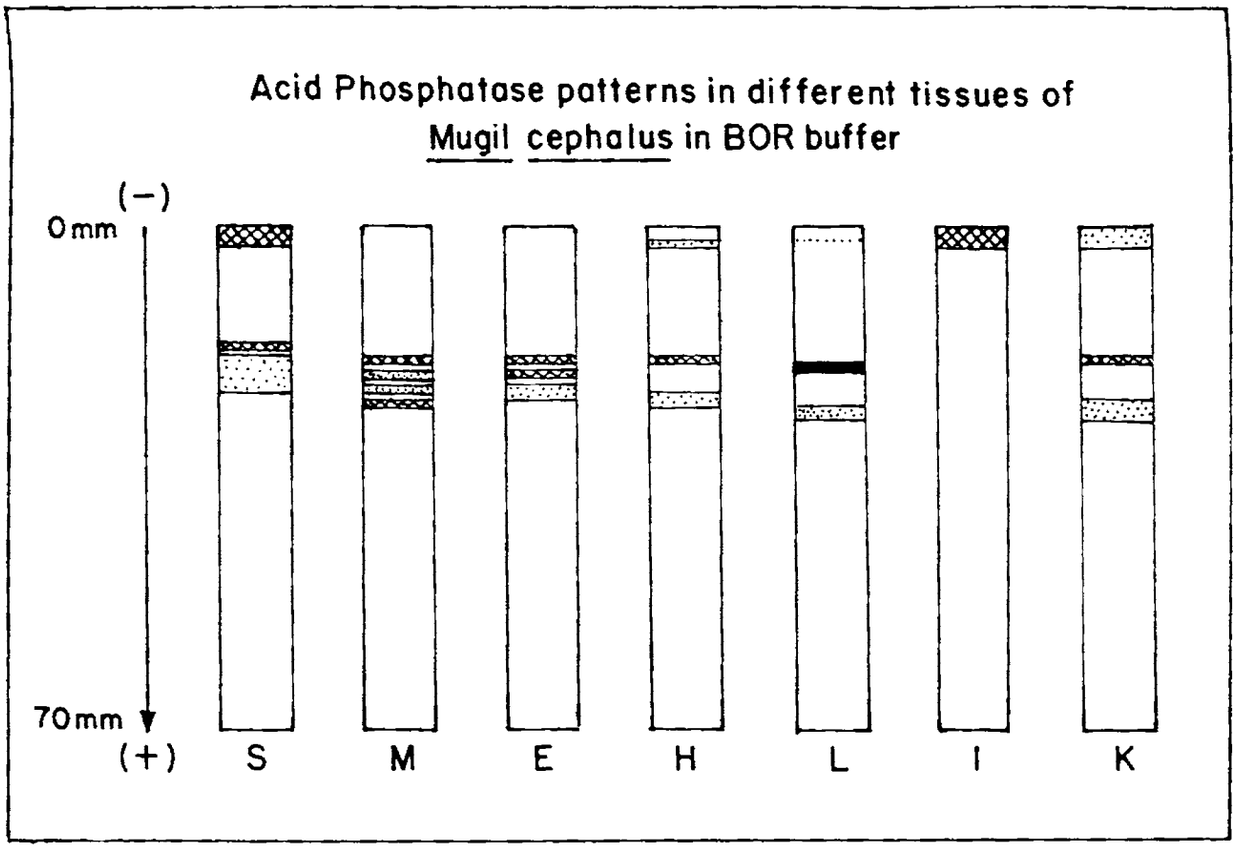

Acid Phosphatase patterns in different tissues of
Mugil cephalus in BOR buffer
(−)
0mm
70mm
(+)
S
M
E
H
L
I
K

FIG. 3

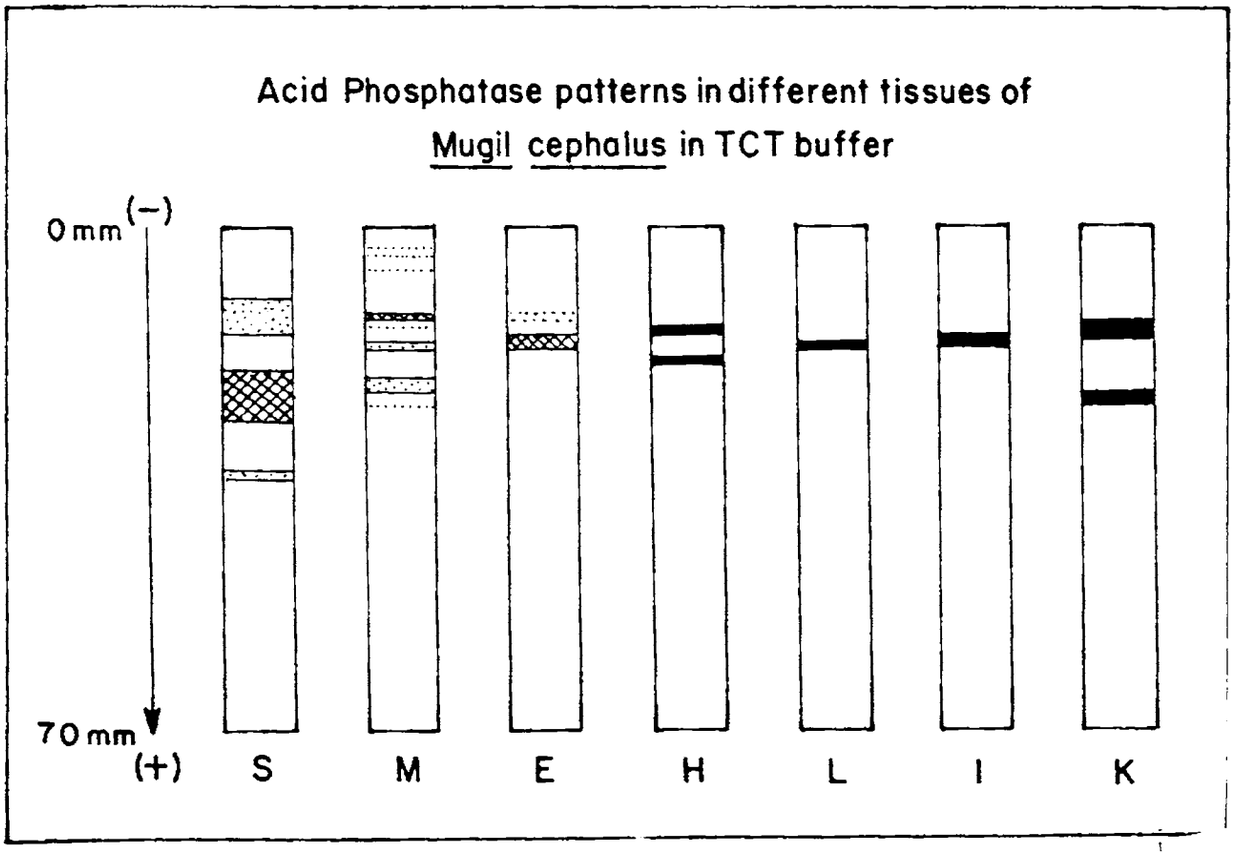

Acid Phosphatase patterns in different tissues of
Mugil cephalus in TCT buffer
0mm
(−)
70mm
(+)
S
M
E
H
L
I
K

FIG. 4

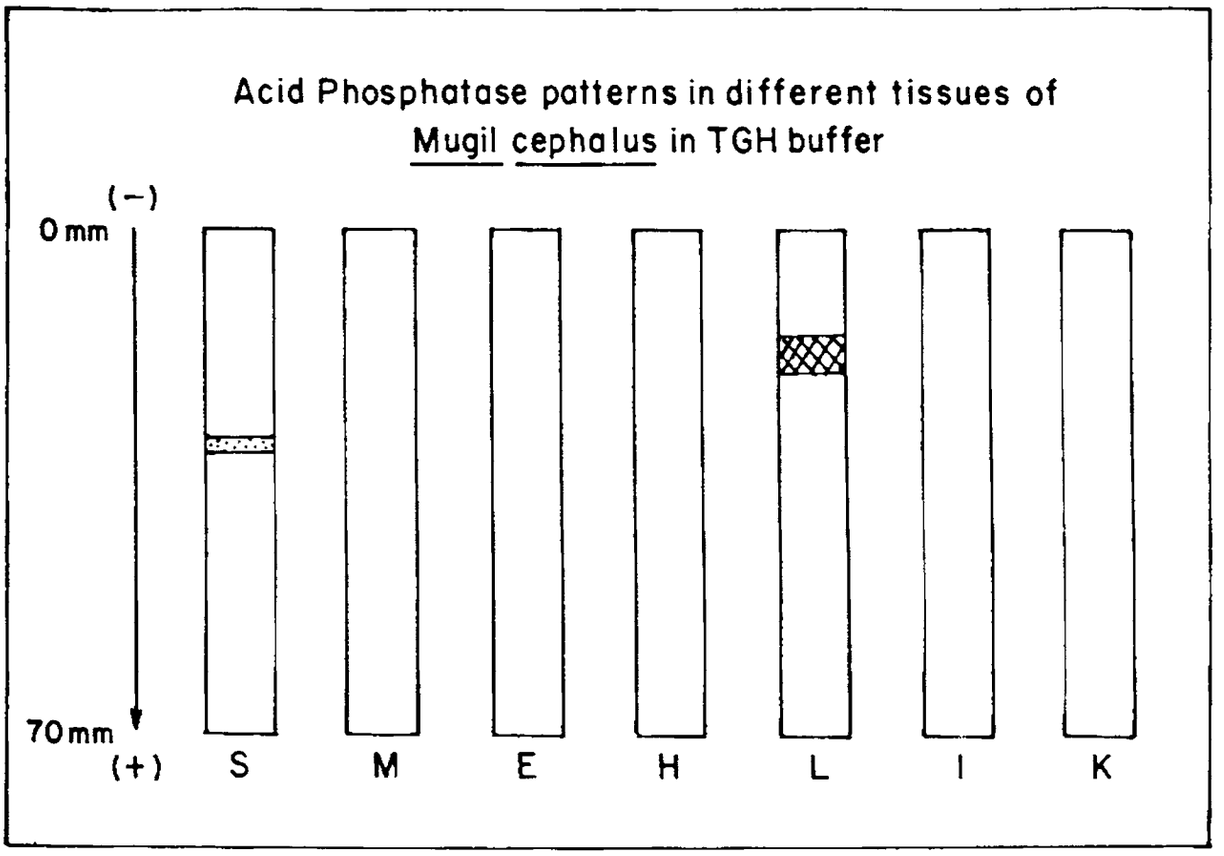

Acid Phosphatase patterns in different tissues of
Mugil cephalus in TGH buffer
(−)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

FIG. 5

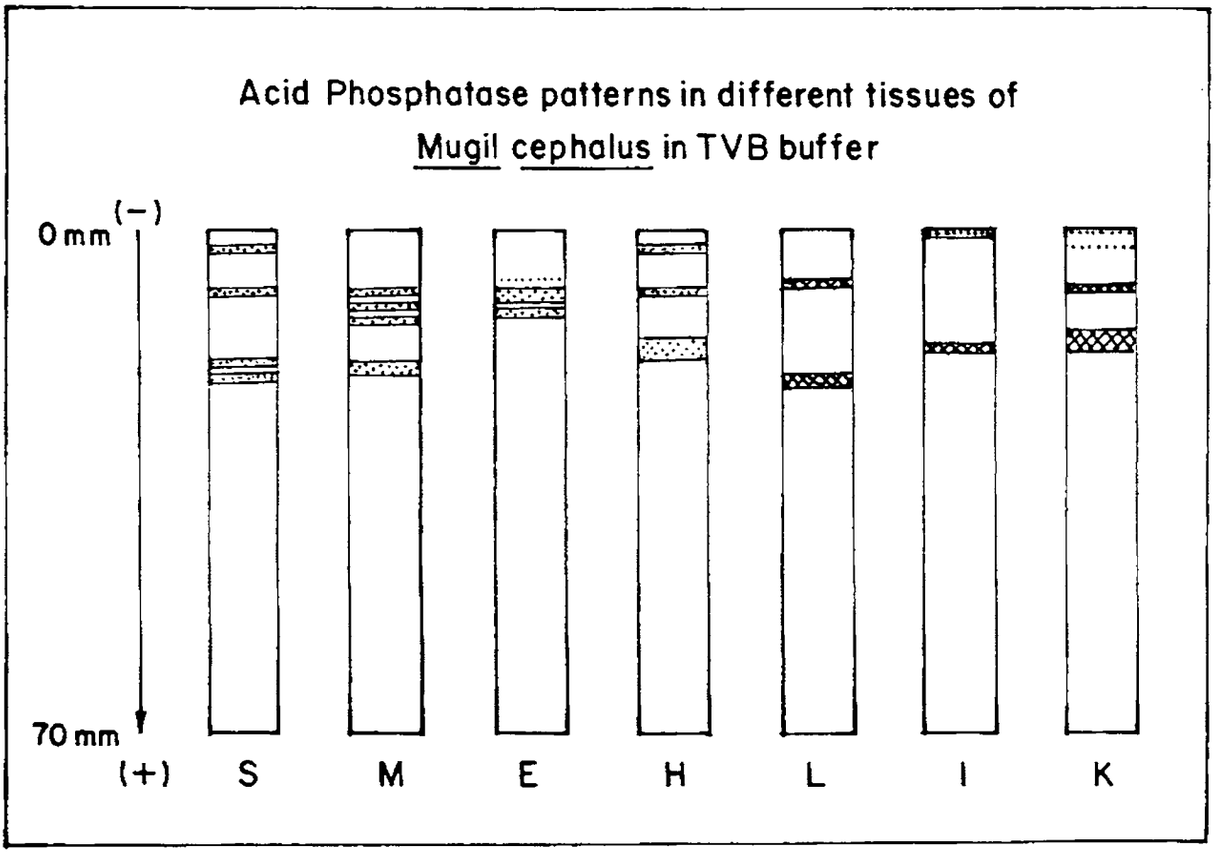

Acid Phosphatase patterns in different tissues of
Mugil cephalus in TVB buffer
(−)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

PLATE 3

Acid Phosphatase patterns in different tissues of *M. cephalus* in

A. Borate buffer

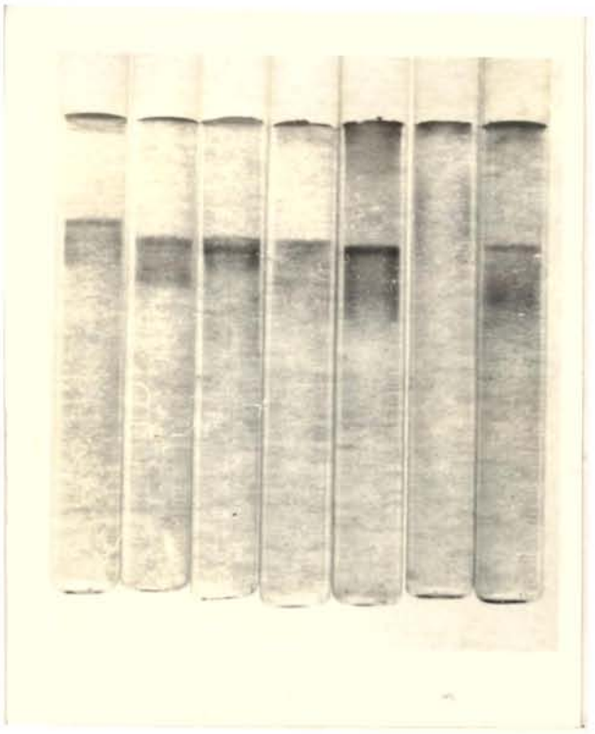
B. Tris Citrate buffer

C. Tris Glycine HCl buffer

D. Tris Versene Borate buffer

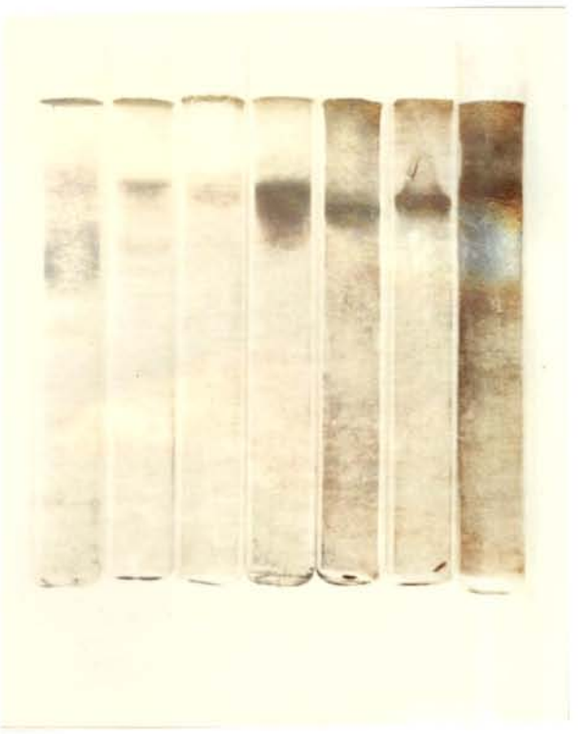
PLATE 3

(A) ACP-BOR



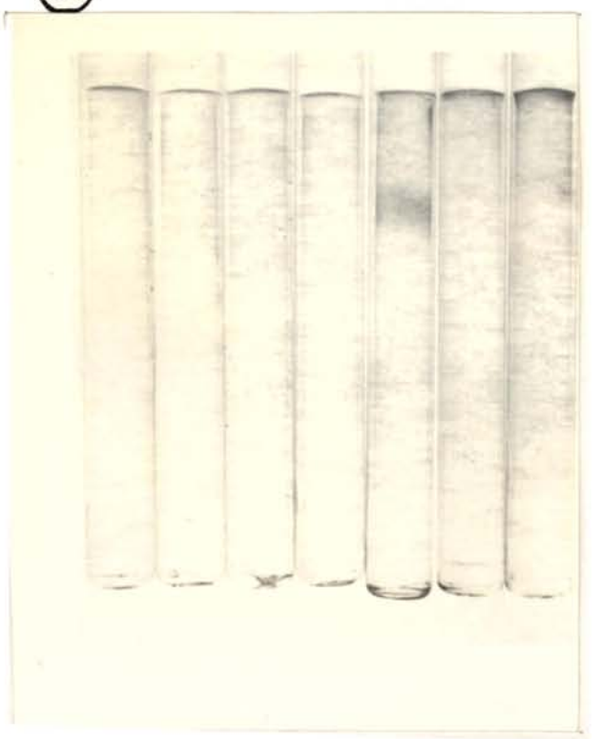
S M E H L I K

(B) ACP-TCT



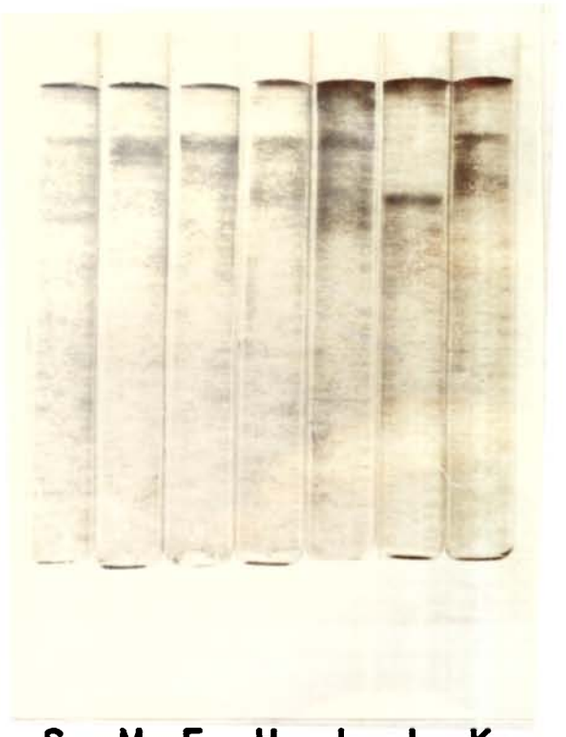
S M E H L I K

(C) ACP-TGH



S M E H L I K

(D) ACP-TVB



S M E H L I K

TABLE 4 *

Experimental Details of Electrophoresis of ACID PHOSPHATASE in

Mugil cephalus L.

Sl. No.	Electrophoretic Buffer Systems **	Tissues	Relative Electrophoretic Mobility (mm) and Intensity (x) of Different Bands								Total No. of Bands	Nature of Separation and Resolution
			1	2	3	4	5	6	7	8		
I	BOR	S	0-3 2x	16-17 2x	18-23 1x						3	P
		M	18-19 2x	20-21 1x	22-23 1x	24-25 2x					4	G
		E	18-19 2x	20-21 2x	22-24 1x						3	G
		H	2-3 1x	18-19 2x	23-25 1x						3	G
		L	2 1x	19-20 3x	25-27 1x						3	B
		I	0-3 2x								1	P
		K	0-3 1x	18-19 2x	24-27 1x						3	P
II	TCT	S	10-15 1x	20-27 2x	34-35 1x						3	P
		M	3 1x	4 1x	6 1x	12-13 2x	14 1x	16-17 1x	21-23 1x	25 1x	8	P
		E	12 1x	13 1x	15-17 2x						3	P
		H	14-15 3x	18-19 3x							2	G
		L	16-17 3x								1	G
		I	15-17 3x								1	G
		K	13-16 3x	23-25 3x							2	P
III	TGH	S	29-31 1x								1	P
		M									0	
		E									0	
		H									0	
		L	15-20 2x								1	G
		I									0	
		K									0	
IV	TVB	S	2-3 1x	8-9 1x	18-19 1x	20-21 1x					4	P
		M	8-9 1x	10-11 1x	12-13 1x	18-20 1x					4	P
		E	7 1x	8-10 1x	11-12 1x						3	P
		H	2-3 1x	8-9 1x	15-18 1x						3	G
		L	7-8 2x	20-22 2x							2	G
		I	0-1 1x	16-17 2x							2	P
		K	1 1x	3 1x	8-9 2x	14-17 2x					4	G

*Refer Page xvi for explanation of abbreviations

**Refer Table 2 for buffer systems.

FIG.6

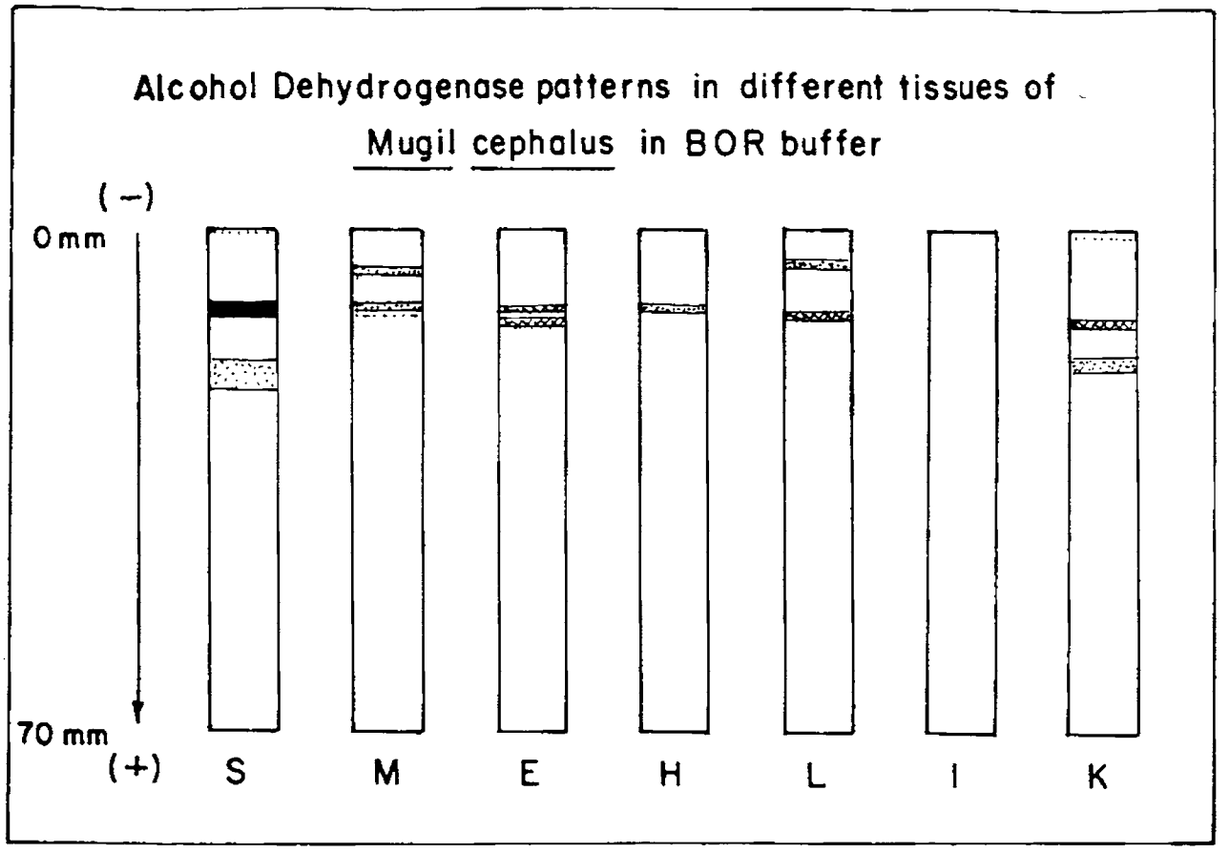

Alcohol Dehydrogenase patterns in different tissues of
Mugil cephalus in BOR buffer
(−)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

FIG.7

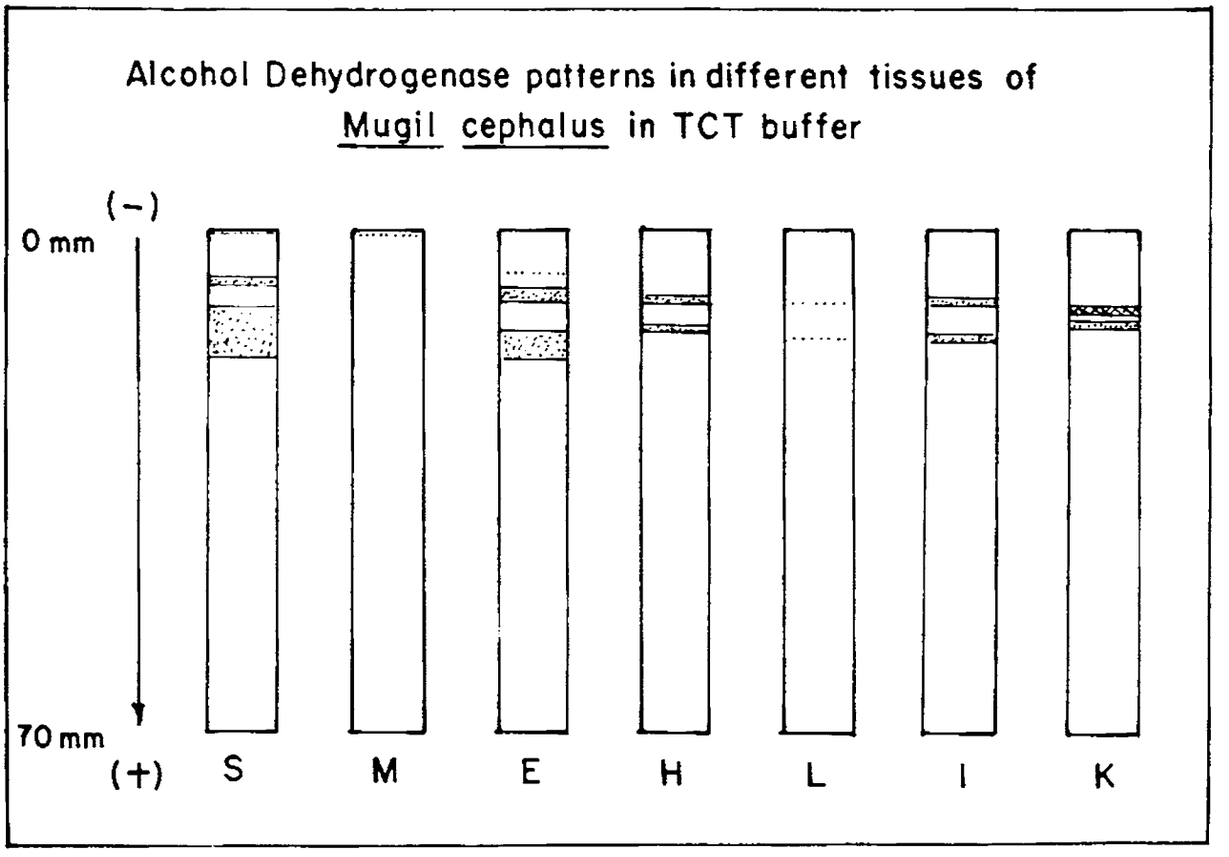

Alcohol Dehydrogenase patterns in different tissues of
Mugil cephalus in TCT buffer
(−)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

FIG. 8

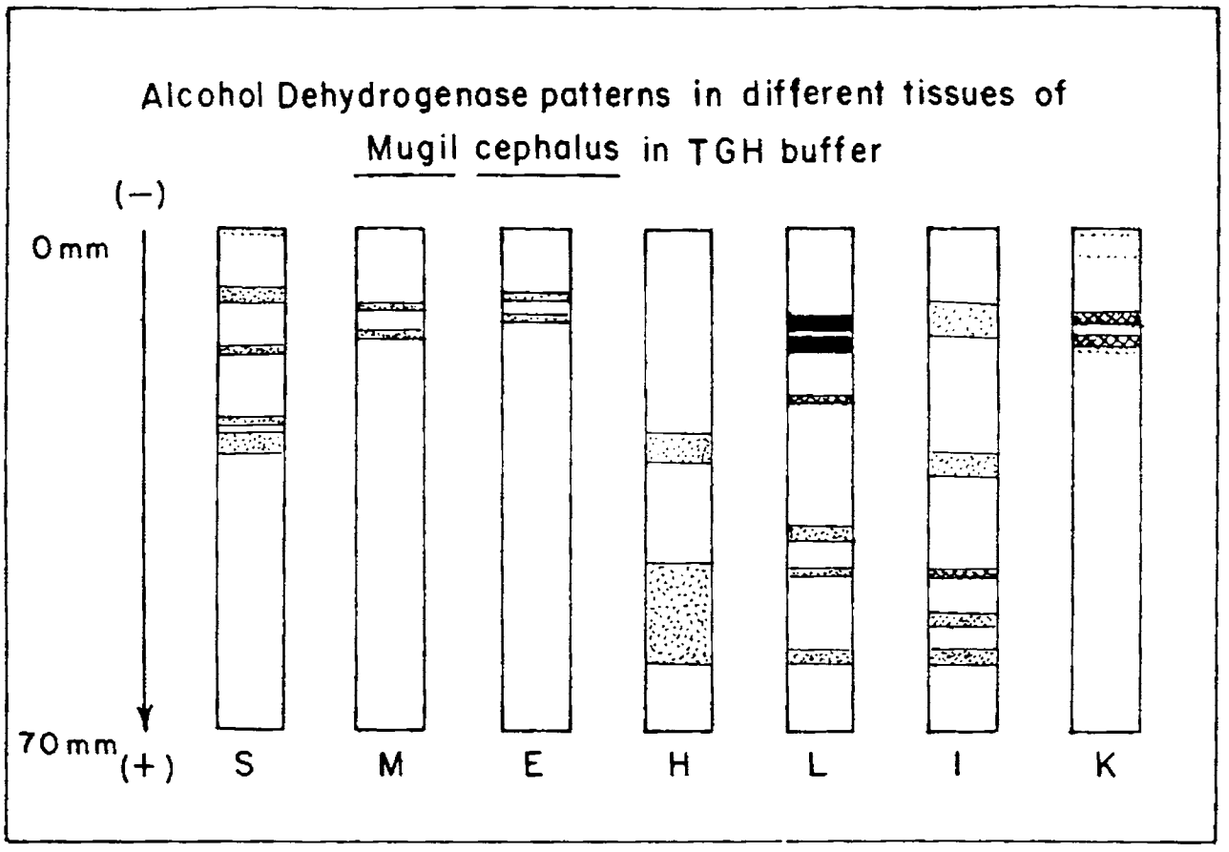

Alcohol Dehydrogenase patterns in different tissues of
Mugil cephalus in TGH buffer
(−)
0mm
70mm
(+)
S
M
E
H
L
I
K

FIG. 9

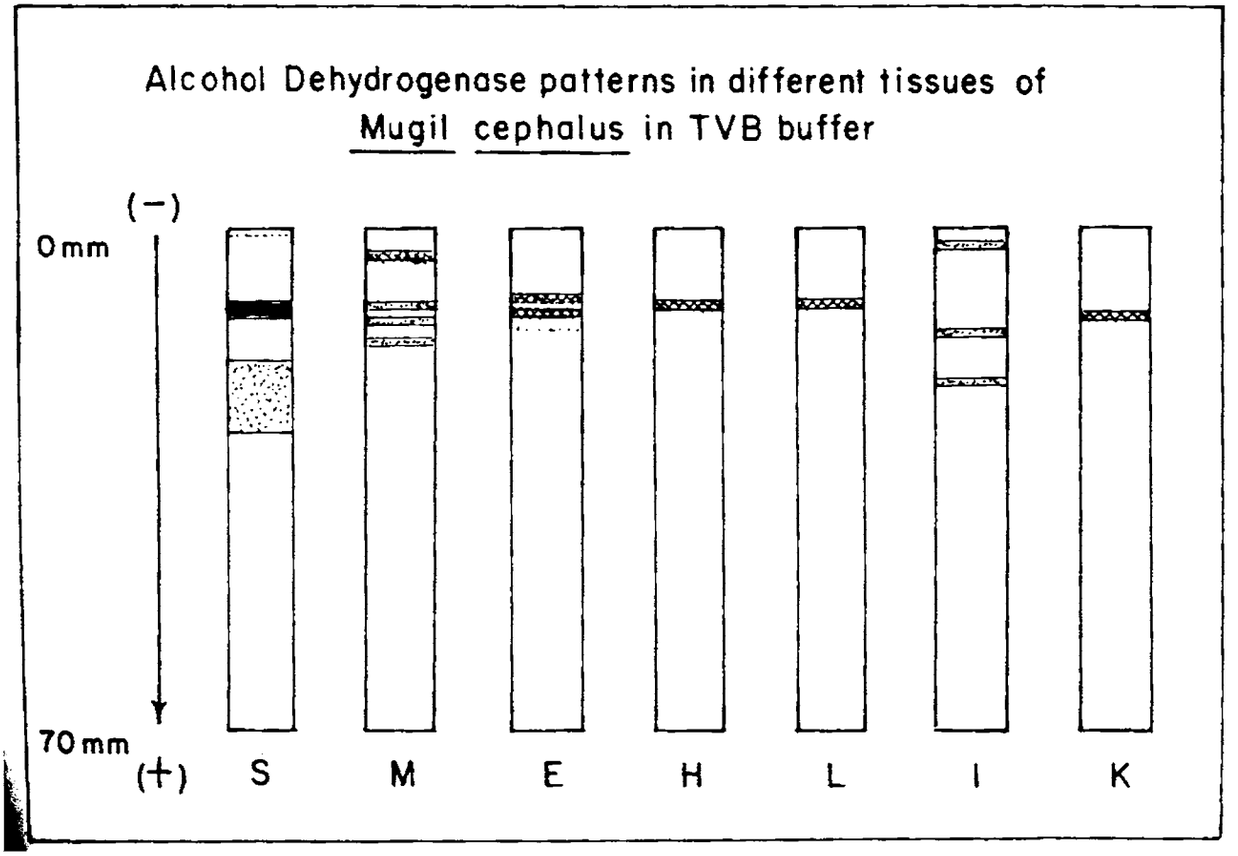

Alcohol Dehydrogenase patterns in different tissues of
Mugil cephalus in TVB buffer
(−)
0mm
70mm
(+)
S
M
E
H
L
I
K

PLATE 4

Alcohol Dehydrogenase patterns in different tissues of M. cephalus in

A. Borate buffer

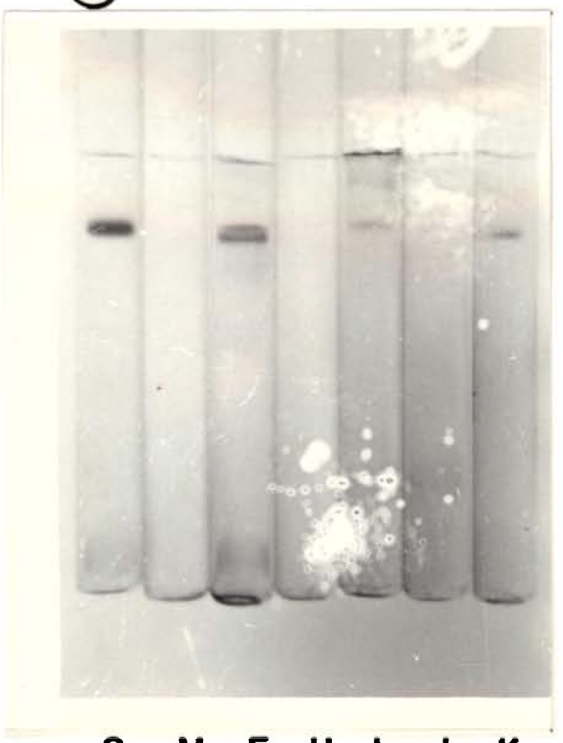
B. Tris Citrate buffer

C. Tris Glycine HCl buffer

D. Tris Versene Borate buffer

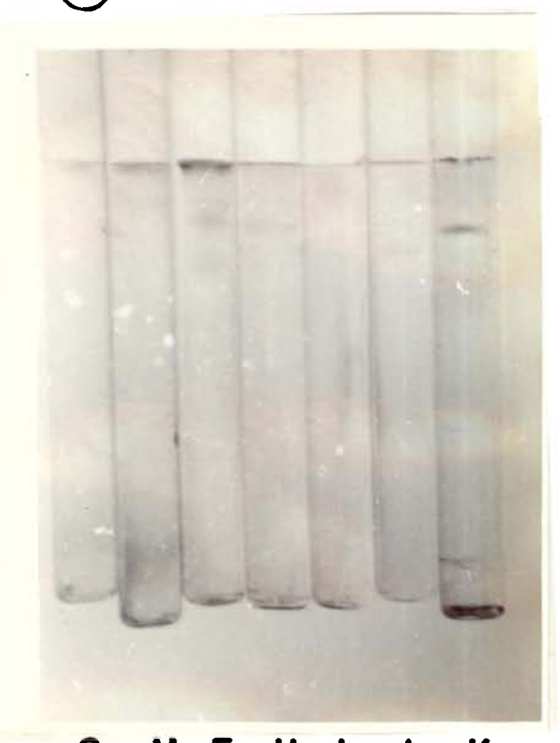
PLATE 4

(A) ADH-BOR



S M E H L I K

(B) ADH-TCT

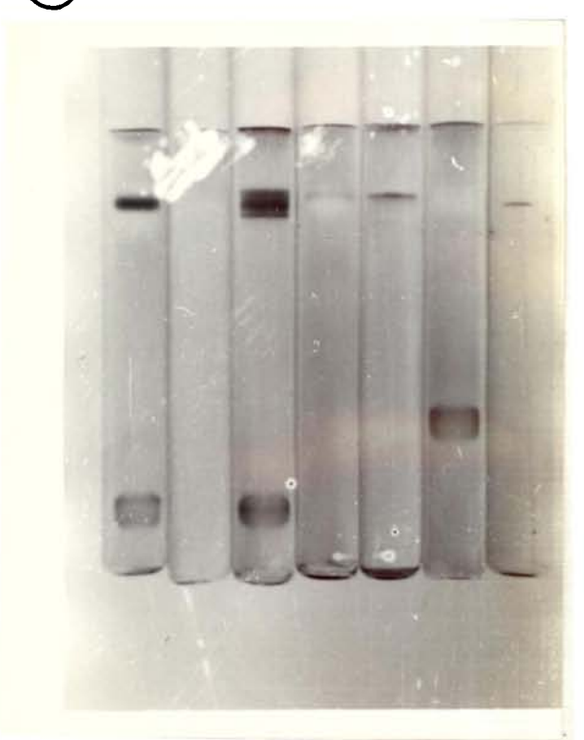


S M E H L I K

(C) ADH-TGH

S M E H L I K

(D) ADH-TVB



S M E H L I K

TABLE 5*

Experimental Details of Electrophoresis of ALCOHOL DEHYDROGENASE in *Mugil cephalus* L.

Sl. No.	Electrophoretic Buffer Systems **	Tissues	Relative Electrophoretic Mobility (mm) and Intensity (x) of Different Bands												Total No.of Bands	Nature of Separation and Resolution
			1		2		3		4		5		6			
I	BOR	S	1	1x	10-12	3x	18-22	1x							3	G
		M	5-6	1x	10-11	1x	12	1x							3	P
		E	10-11	2x	12-13	2x									2	G
		H	10-11	1x											1	P
		L	4-5	1x	11-12	2x									2	G
		I													0	
		K	1	1x	12-13	2x	17-19	1x							3	P
II	TCT	S	1	1x	7-8	1x	11-18	1x							3	P
		M	1	1x											1	P
		E	6	1x	8-10	1x	14-18	1x							3	P
		H	9-10	1x	13-14	1x									2	P
		L	10	1x	15	1x									2	P
		I	9-10	1x	14-15	1x									2	P
		K	10-11	2x	12-13	1x									2	P
III	TGH	S	1	1x	8-10	1x	16-17	1x	26-27	1x	28-31	1x			5	P
		M	10-11	1x	14-15	1x									2	P
		E	9-10	1x	12-14	1x									2	P
		H	28-32	1x	46-60	1x									2	P
		L	12-14	3x	15-17	3x	23-24	2x	41-43	1x	47-48	1x	58-60	1x	6	B
		I	10-15	1x	31-34	1x	47-48	2x	53-55	1x	58-60	1x			5	P
		K	1	1x	4	1x	12-13	2x	15-16	2x	17	1x			5	G
IV	TVB	S	1	1x	10-12	3x	18-23	1x							3	G
		M	3-4	2x	10-11	1x	12-13	1x	15-16	1x					4	P
		E	9-10	2x	11-12	2x	14	1x							3	G
		H	10-11	2x											1	P
		L	10-11	2x											1	G
		I	2-3	1x	14-15	1x	21-22	1x							3	P
		K	12-13	2x											1	G

* Refer Page xvi for explanation of abbreviations

** Refer Table 2 for buffer systems

FIG. 10

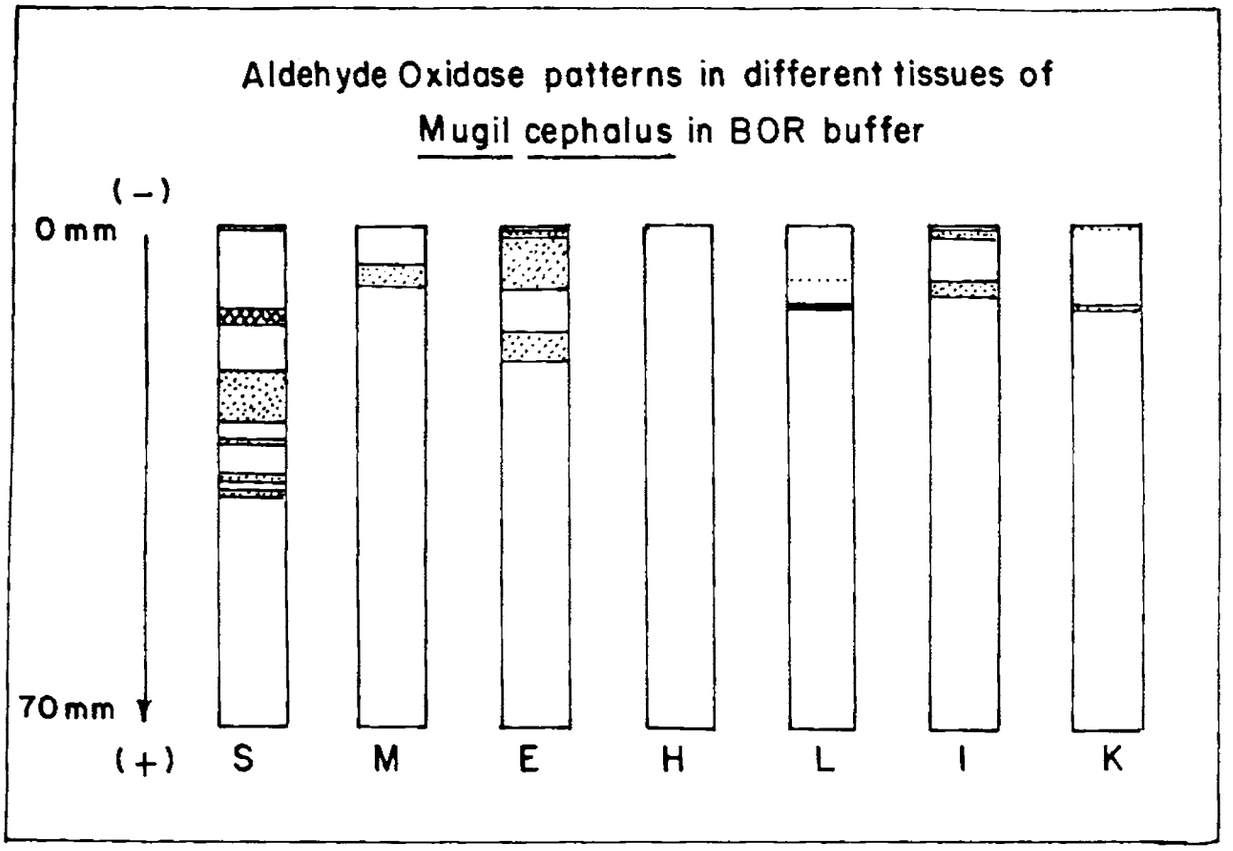

Aldehyde Oxidase patterns in different tissues of
Mugil cephalus in BOR buffer
(−)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

FIG. 11

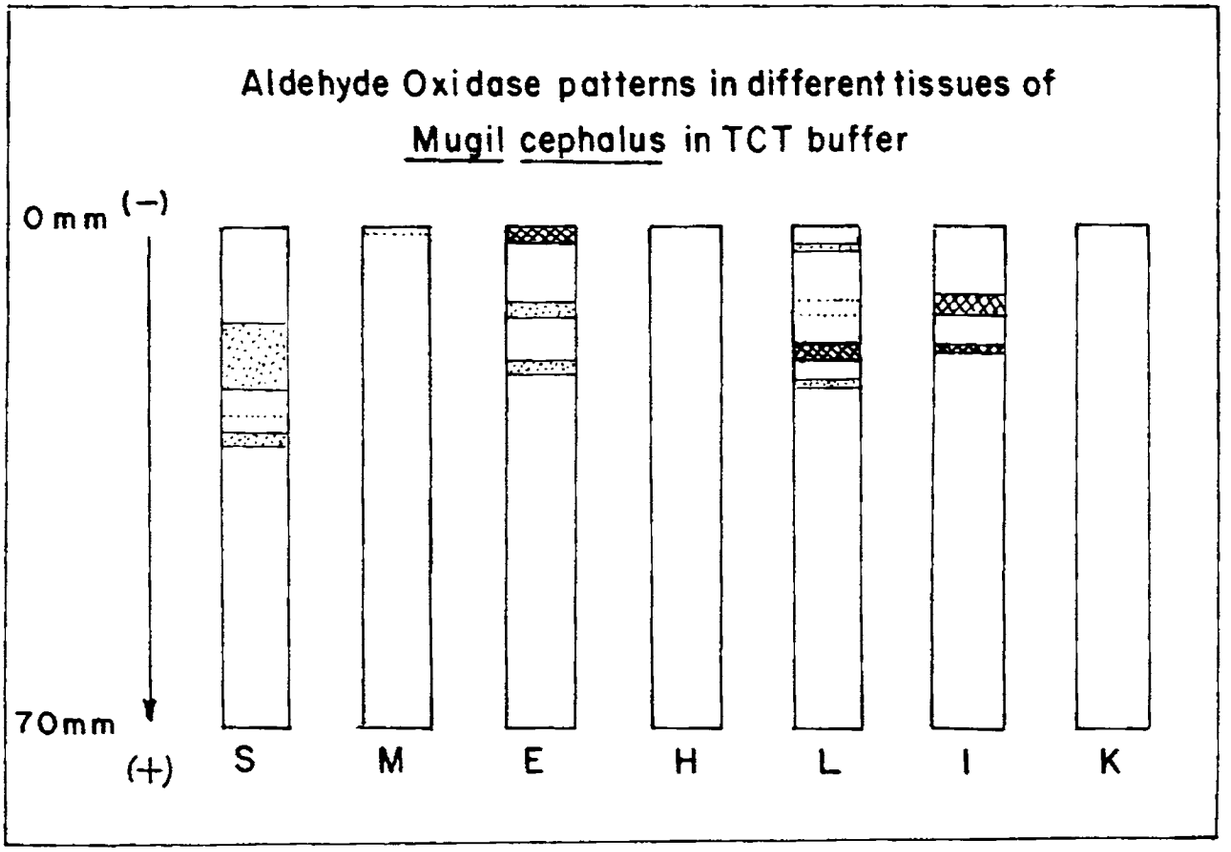

Aldehyde Oxidase patterns in different tissues of
Mugil cephalus in TCT buffer
0 mm
(−)
70 mm
(+)
S
M
E
H
L
I
K

FIG. 12

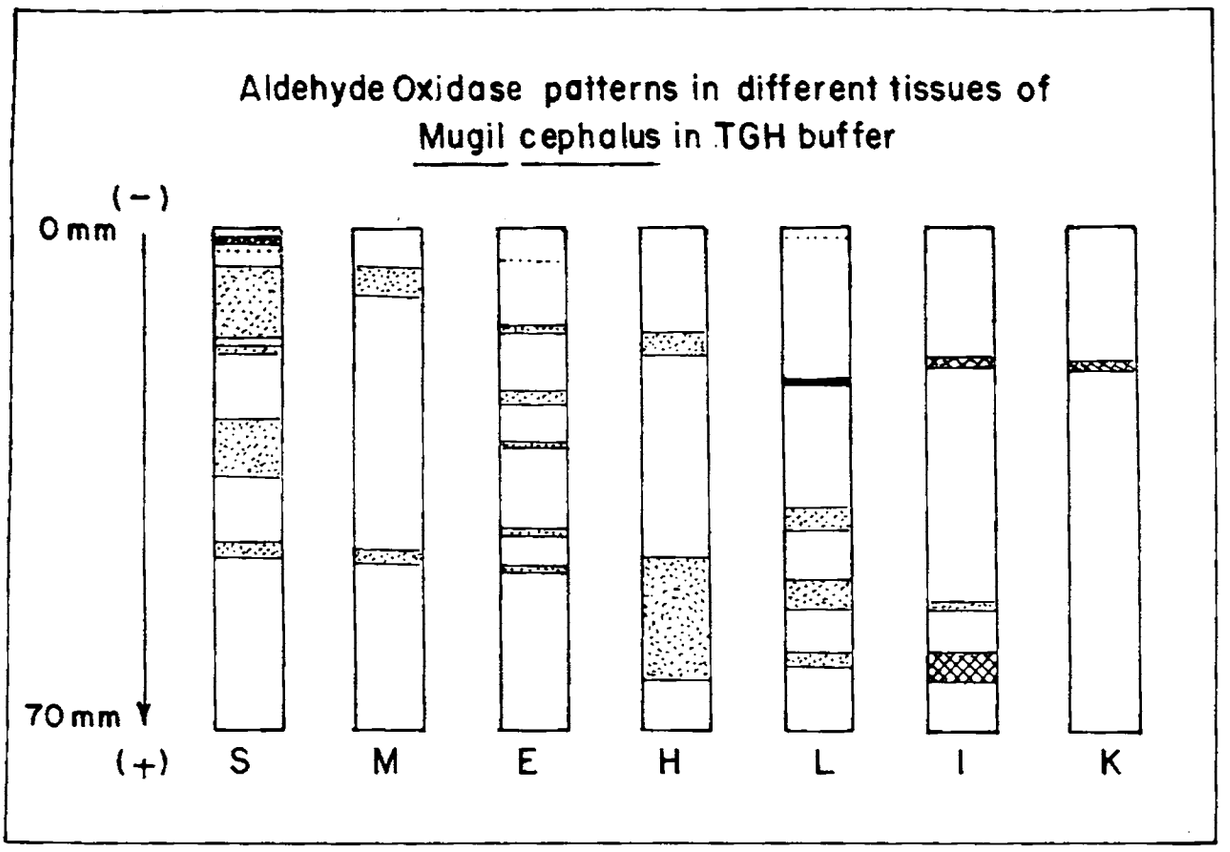

Aldehyde Oxidase patterns in different tissues of
Mugil cephalus in TGH buffer
(−)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

FIG. 13

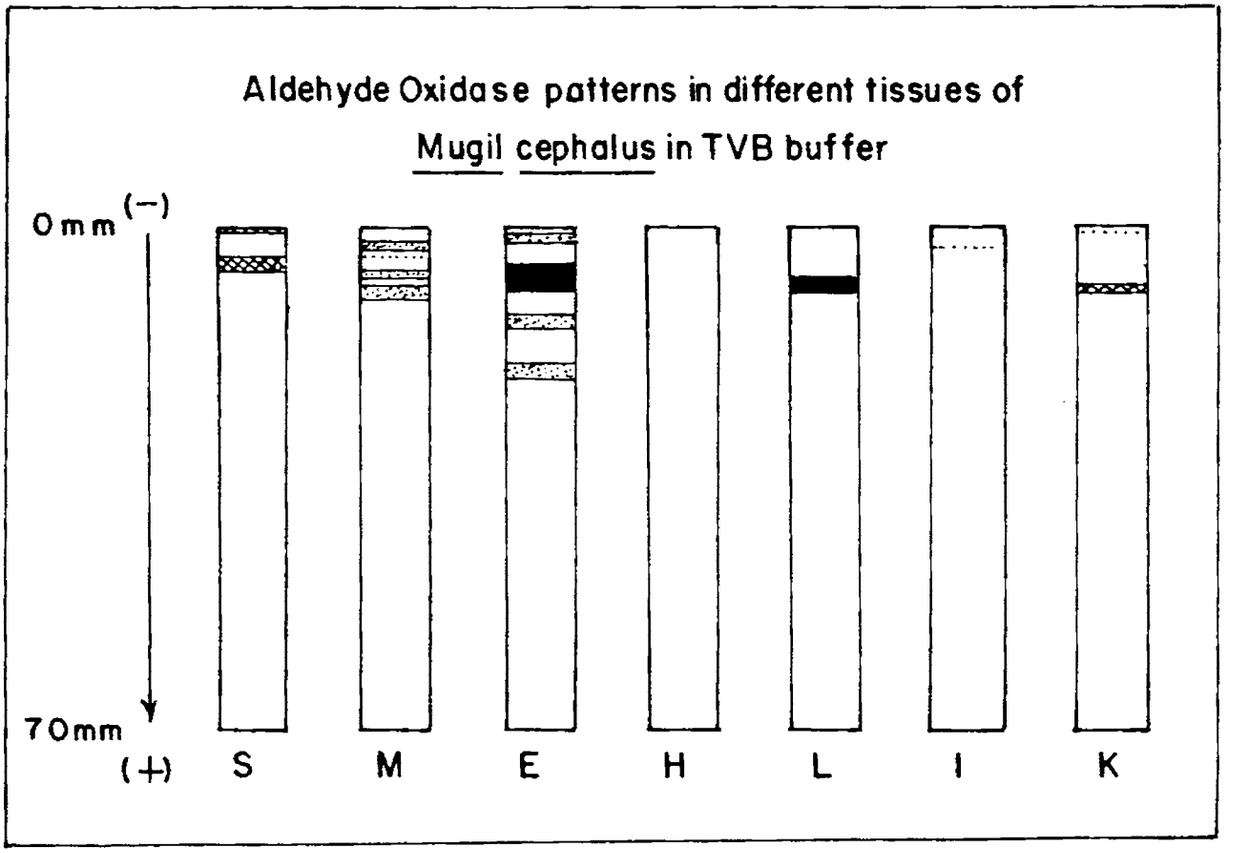

Aldehyde Oxidase patterns in different tissues of
Mugil cephalus in TVB buffer
0 mm
(−)
70 mm
(+)
S
M
E
H
L
I
K

PLATE 5

Aldehyde Oxidase patterns in different tissues of *M. cephalus* in

A. Borate buffer

B. Tris Citrate buffer

C. Tris Glycine HCl buffer

C. Tris Versene Borate buffer

PLATE 5

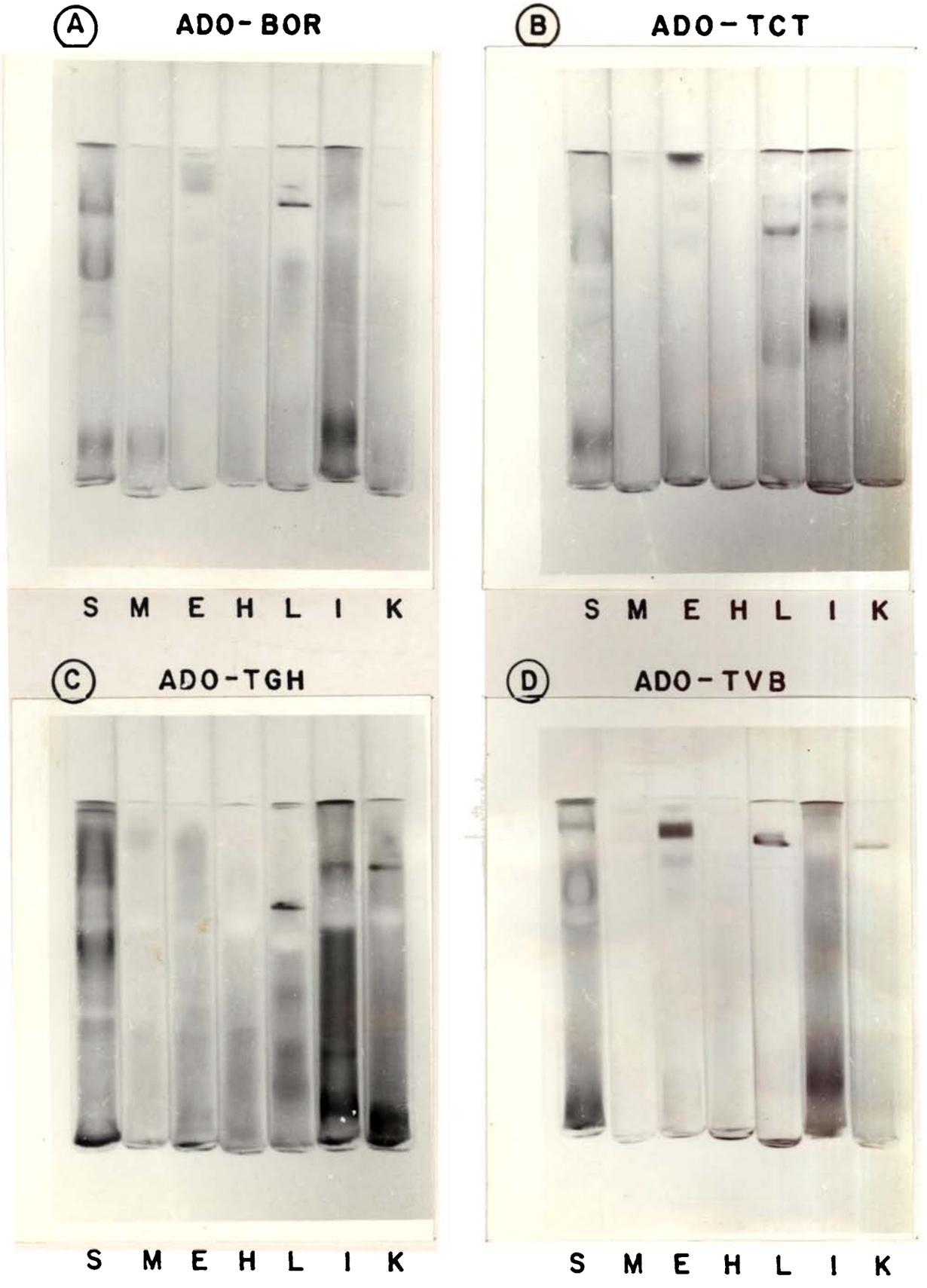

A
ADO-BOR
S M E H L I K
B
ADO-TCT
S M E H L I K
C
ADO-TGH
S M E H L I K
D
ADO-TVB
S M E H L I K

TABLE 6 *

Experimental Details of Electrophoresis of ALDEHYDE OXIDASE in *Mugil cephalus* L.

Sl. No.	Electrophoretic Buffer Systems **	Tissues	Relative Electrophoretic Mobility (mm) and Intensity (x) of Different Bands												Total No.of Bands	Nature of Separation and Resolution
			1		2		3		4		5		6			
I	BOR	S	0-1	1x	12-14	2x	21-28	1x	30-31	1x	35-36	1x	37-38	1x	6	P
		M	6-9	1x											1	P
		E	0-1	1x	2-9	1x	15-19	1x							3	P
		H													0	
		L	8	1x	11-12	3x									2	G
		I	1-2	1x	8-11	1x									2	P
		K	1	1x	11-12	1x									2	P
II	TCT	S	13-22	1x	26	1x	28-30	1x							3	P
		M	1	1x											1	P
		E	0-2	2x	10-12	1x	18-20	1x							3	P
		H													0	
		L	2-3	1x	10	1x	12	1x	16-18	2x	21-22	1x			5	G
		I	9-12	2x	16-17	2x									2	G
		K													0	
III	TGH	S	2-3	2x	4	1x	5-15	1x	16-17	1x	26-34	1x	43-45	1x	6	G
		M	5-9	1x	44-46	1x									2	P
		E	4	1x	13-14	1x	23-24	1x	29-30	1x	41-42	1x	46-47	1x	6	P
		H	14-17	1x	45-62	1x									2	P
		L	1	1x	21-22	3x	38-41	1x	48-52	1x	58-60	1x			5	B
		I	18-19	2x	51-52	1x	58-62	2x							3	G
		K	18-19	2x											1	G
IV	TVB	S	0-1	2x	4-6	2x									2	G
		M	2-3	1x	4	1x	6-7	1x	8-10	1x					4	P
		E	1-2	1x	5-9	3x	12-14	1x	19-21	1x					4	G
		H													0	
		L	7-9	3x											1	G
		I	3	1x											1	P
		K	1	1x	8-9	2x									2	G

* Refer Page xvi for explanation of abbreviations

** Refer Table 2 for buffer systems

FIG. 14

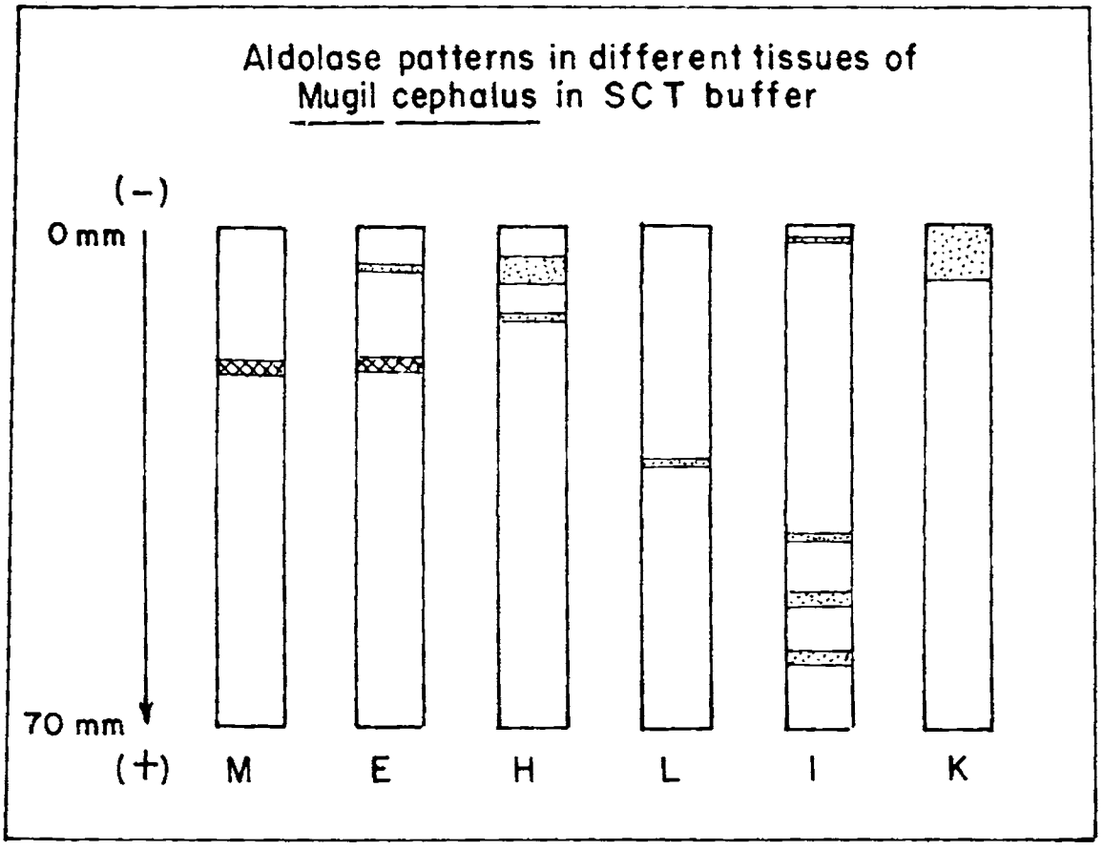

Aldolase patterns in different tissues of
Mugil cephalus in SCT buffer
(−)
0 mm
70 mm
(+)
M
E
H
L
I
K

FIG. 15

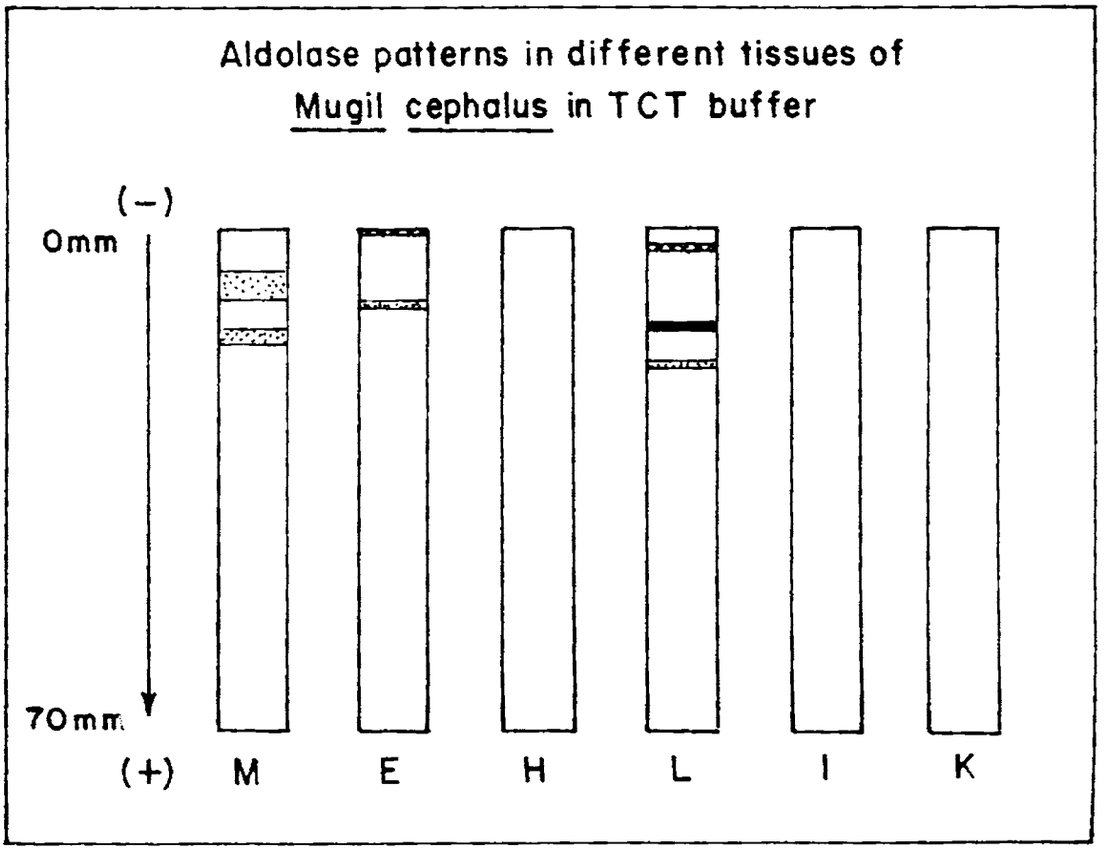

Aldolase patterns in different tissues of
Mugil cephalus in TCT buffer
(−)
0mm
70mm
(+)
M
E
H
L
I
K

FIG. 16

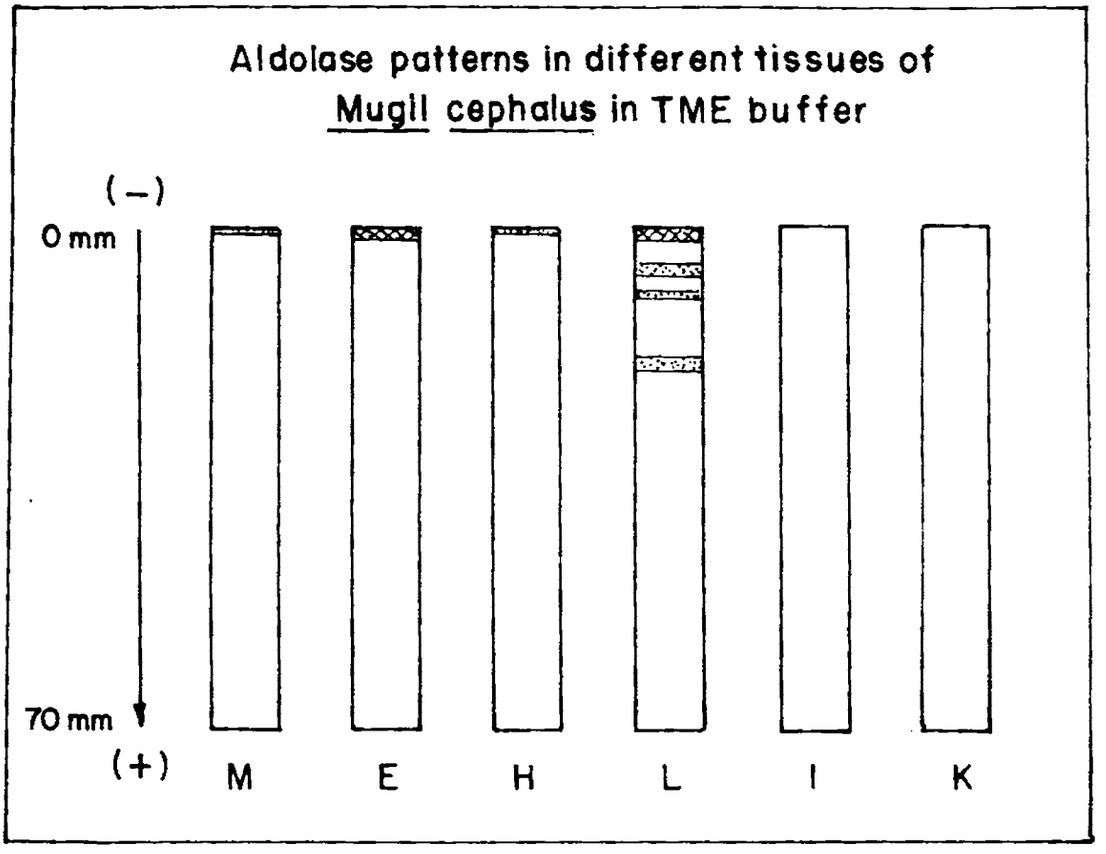

Aldolase patterns in different tissues of
Mugil cephalus in TME buffer
(-)
0 mm
70 mm
(+)
M
E
H
L
I
K

FIG. 17

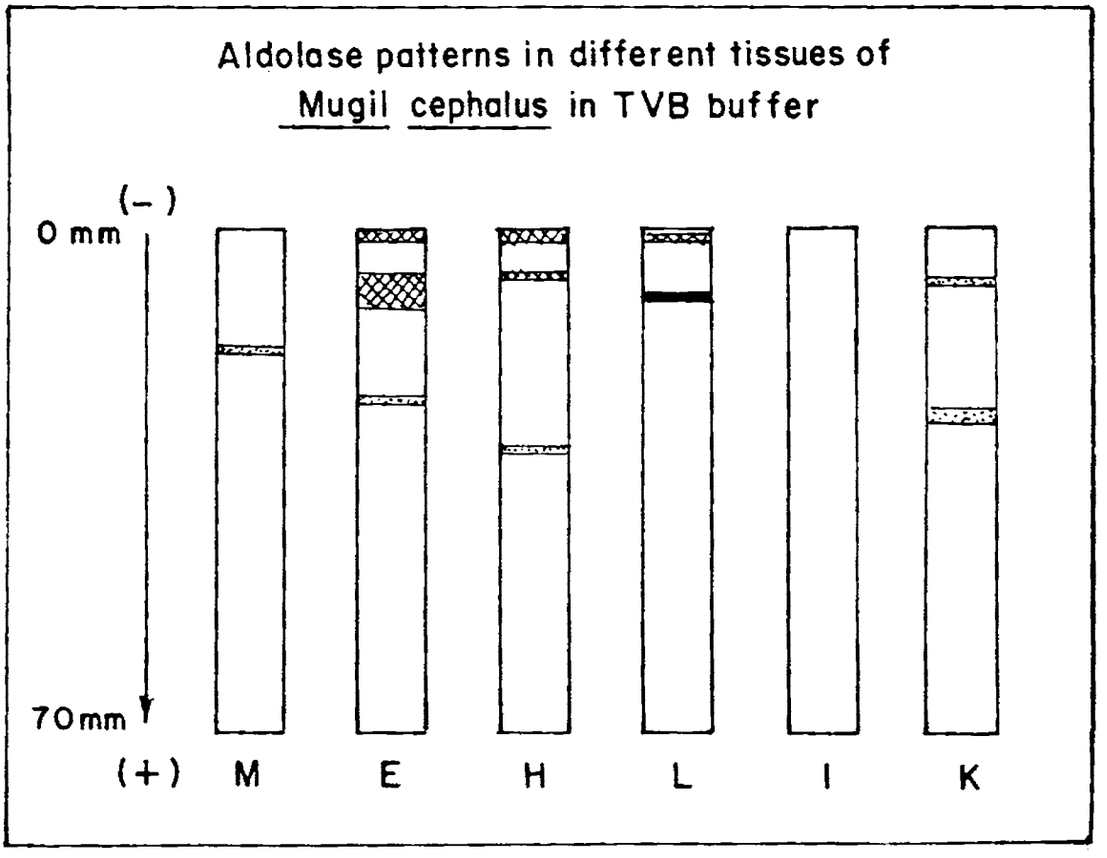

Aldolase patterns in different tissues of
Mugil cephalus in TVB buffer
(-)
0 mm
70 mm
(+)
M
E
H
L
I
K

PLATE 6

Aldolase patterns in different tissues of *M. cephalus* in

A. Borate buffer

B. Tris Citrate buffer

C. Tris Maleic EDTA buffer

C. Tris Versene Borate buffer

PLATE 6

(A) ALD-SCT

M E H L I K

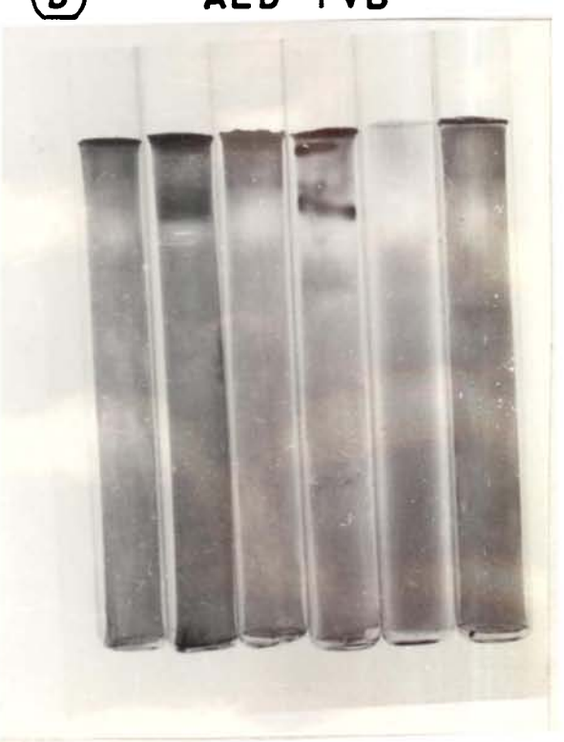
(B) ALD-TCT

M E H L I K

(C) ALD-TME

M E H L I K

(D) ALD-TVB



M E H L I K

TABLE 7*

Experimental Details of Electrophoresis of ALDOLASE in *Mugil cephalus* L.

Sl. No.	Electrophoretic Buffer Systems **	Tissues	Relative Electrophoretic Mobility (mm) and Intensity (x) of Different Bands								Total No. of Bands	Nature of Separation and Resolution
			1		2		3		4			
I	SCT	M	18-20	2x							1	G
		E	5-6	1x	18-20	2x					2	G
		H	4-8	1x	12-13	1x					2	P
		L	32-33	1x							1	P
		I	1-2	1x	42-43	1x	50-52	1x	58-60	1x	4	P
		K	0-7	1x							1	P
II	TCT	M	6-10	1x	14-16	1x					2	G
		E	0-1	2x	10-11	1x					2	G
		H									0	
		L	2-3	2x	13-14	3x	18-19	1x			3	G
		I									0	
		K									0	
III	TME	M	0-1	1x							1	P
		E	0-2	2x							1	G
		H	0-1	1x							1	P
		L	0-2	2x	5-7	1x	9-10	1x	18-20	1x	4	G
		I									0	
		K									0	
IV	TVB	M	16-17	1x							1	P
		E	0-2	2x	6-11	2x	23-24	1x			3	G
		H	0-2	2x	6-7	2x	30-31	1x			3	G
		L	1-2	2x	9-10	3x					2	B
		I									0	
		K	7-8	1x	25-27	1x					2	G

* Refer Page xvi for explanation of abbreviations

** Refer Table 2 for buffer systems

FIG. 18

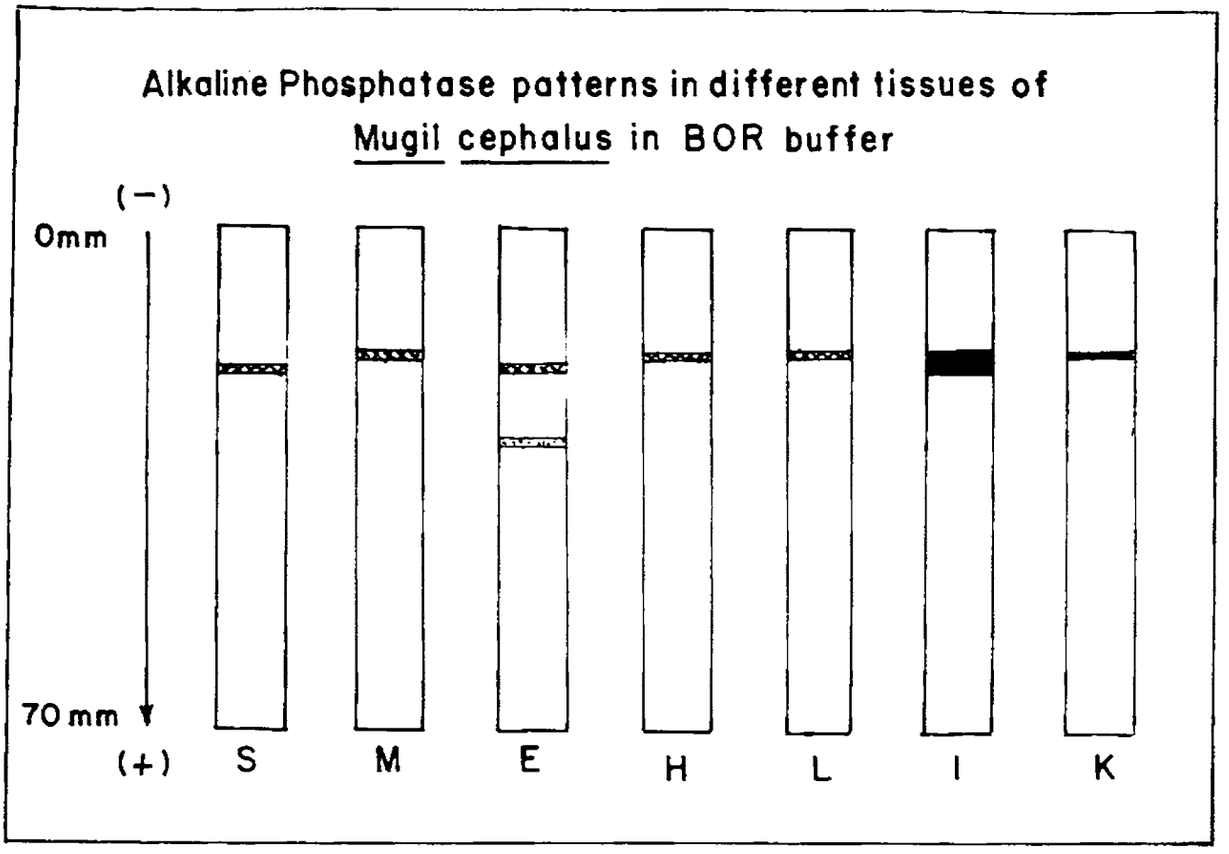

Alkaline Phosphatase patterns in different tissues of
Mugil cephalus in BOR buffer
(−)
0mm
70mm
(+)
S
M
E
H
L
I
K

FIG. 19

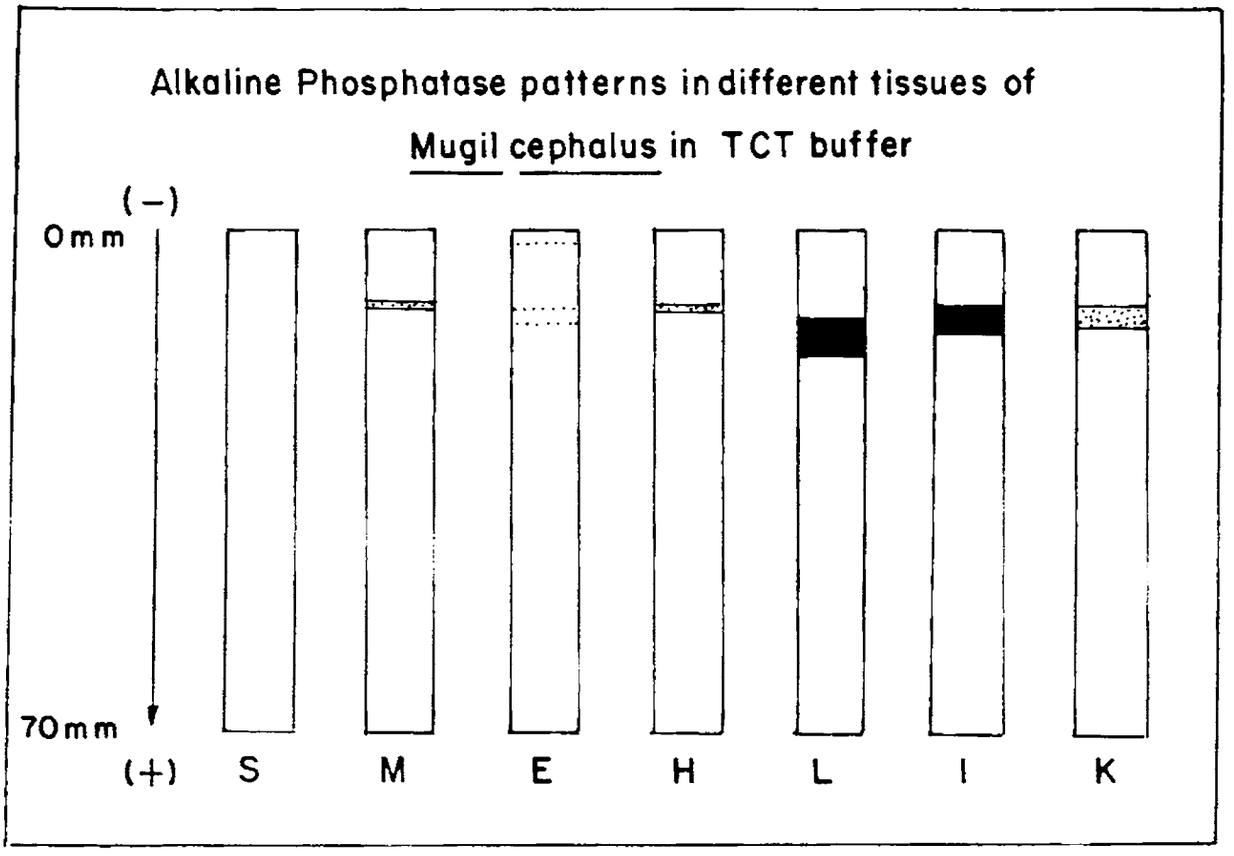

Alkaline Phosphatase patterns in different tissues of
Mugil cephalus in TCT buffer
(−)
0mm
70mm
(+)
S
M
E
H
L
I
K

FIG. 20

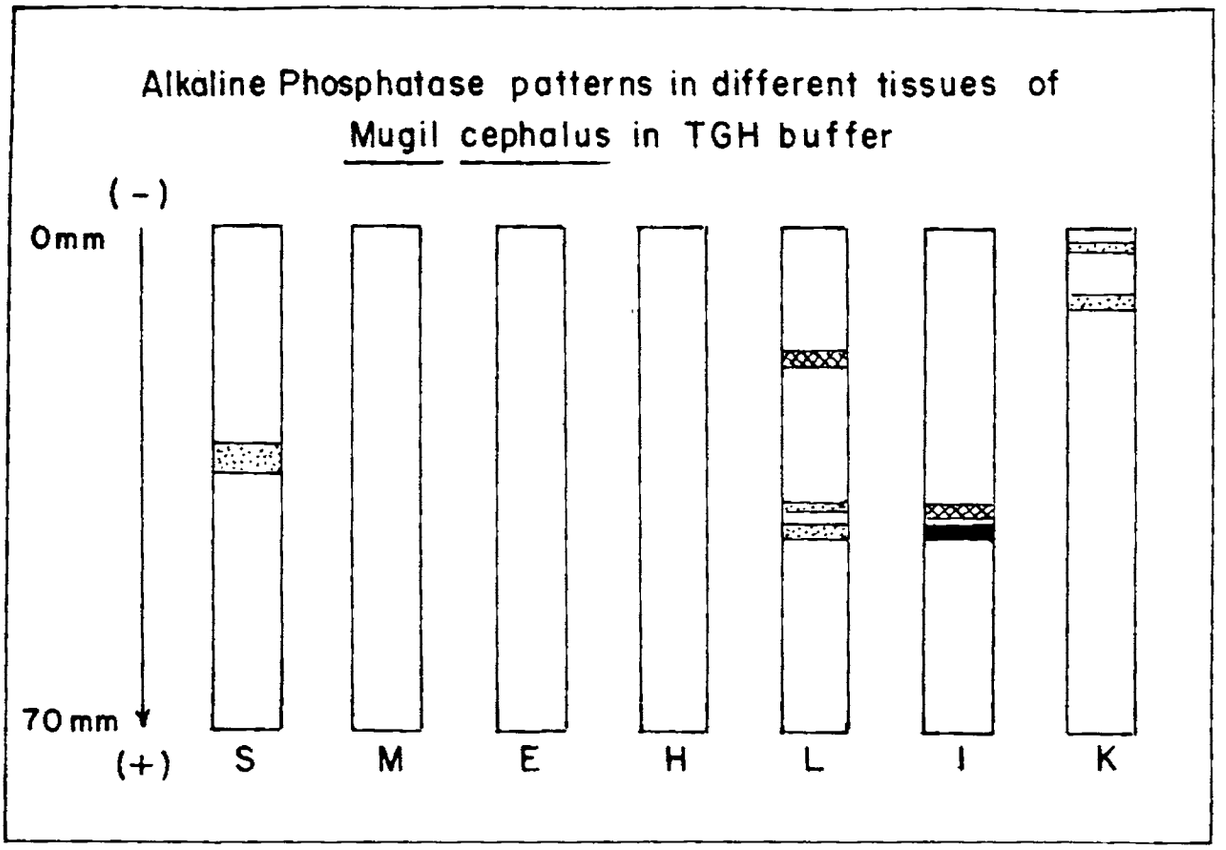

Alkaline Phosphatase patterns in different tissues of
Mugil cephalus in TGH buffer
(-)
0mm
70mm
(+)
S
M
E
H
L
I
K

FIG. 21

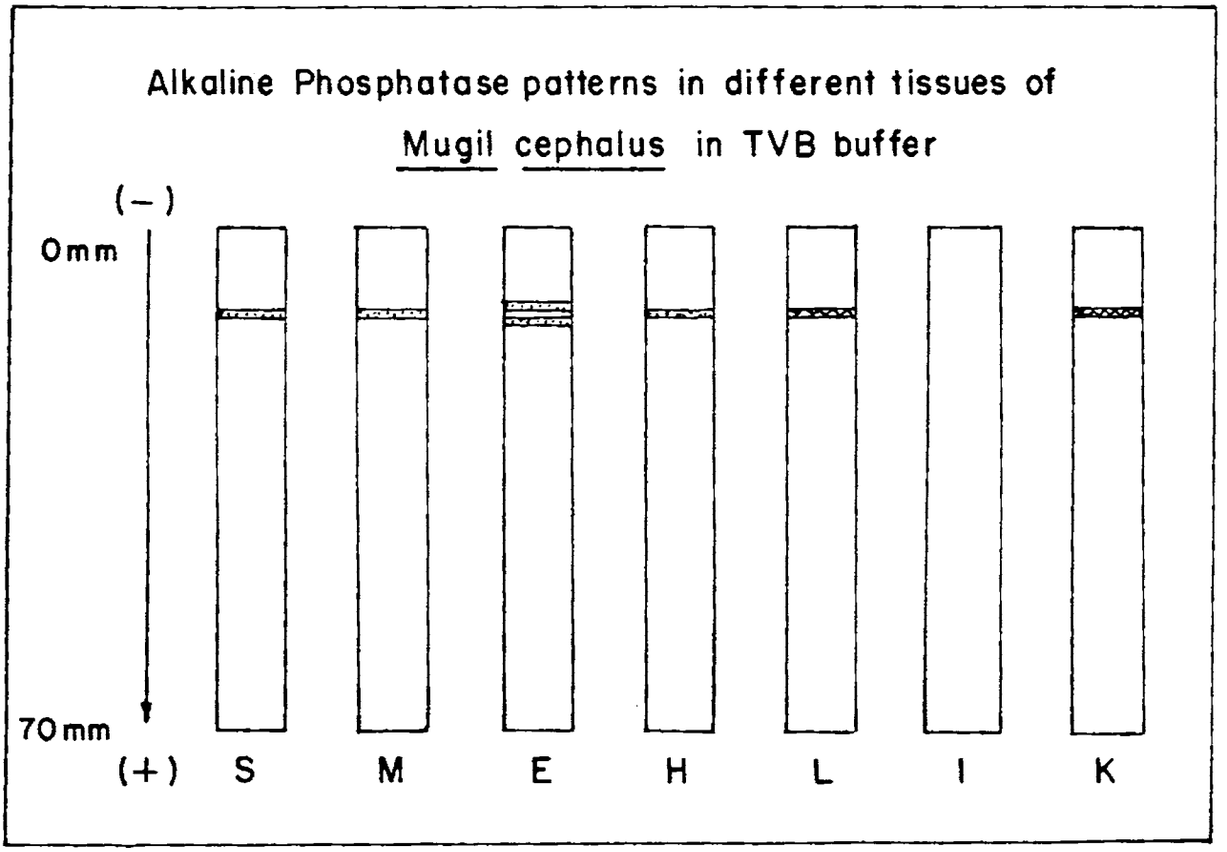

Alkaline Phosphatase patterns in different tissues of
Mugil cephalus in TVB buffer
(-)
0mm
70mm
(+)
S
M
E
H
L
I
K

PLATE 7

Alkaline Phosphatase patterns in different tissues of M. cephalus in

A. Borate buffer

B. Tris Citrate buffer

C. Tris Glycine HCl buffer

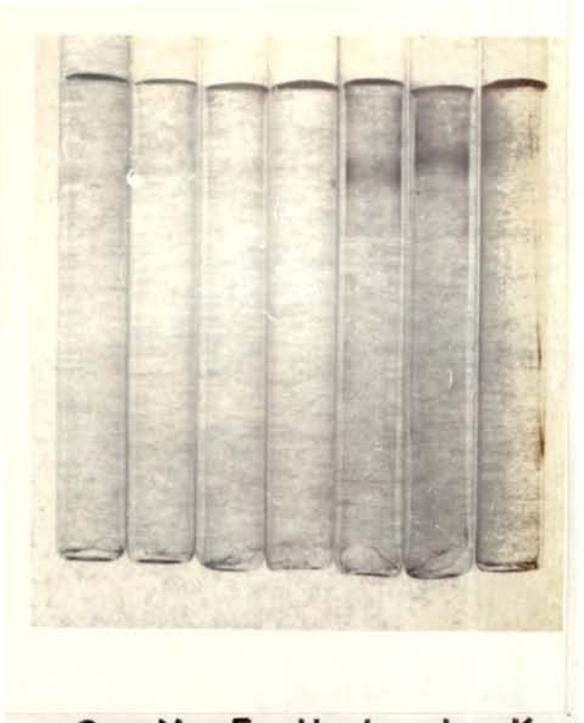
C. Tris Versene Borate buffer

PLATE 7

(A) AKP-BOR

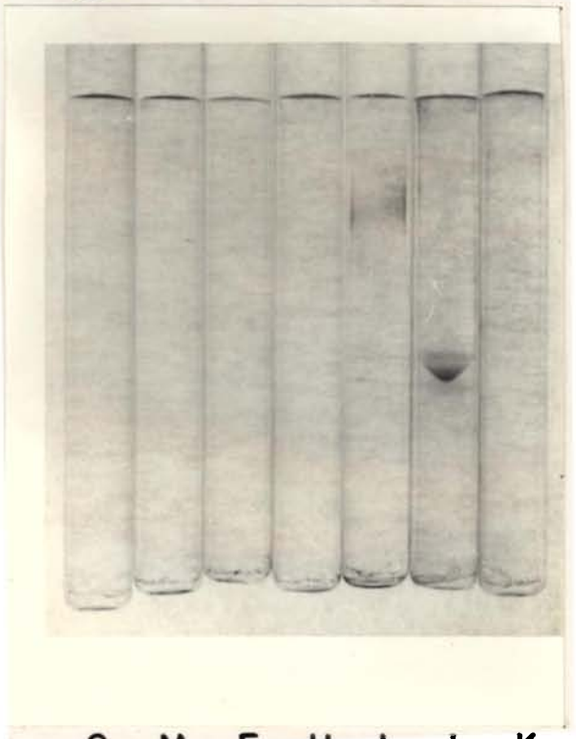
S M E H L I K

(B) AKP-TCT



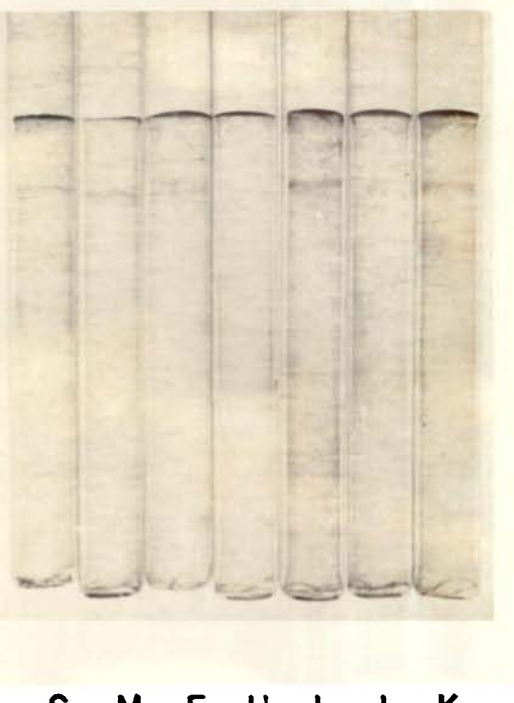
S M E H L I K

(C) AKP-TGH



S M E H L I K

(D) AKP-TVB



S M E H L I K

TABLE 8 *

Experimental Details of Electrophoresis of ALKALINE PHOSPHATASE in *Mugil cephalus* L.

Sl. No.	Electrophoretic Buffer Systems **	Tissues	Relative Electrophoretic Mobility (mm) and Intensity (x) of Different Bands						Total No. of Bands	Nature of Separation and Resolution
			1		2		3			
I	BOR	S	19-20	2x					1	P
		M	17-18	2x					1	P
		E	19-20	2x	29-30	1x			2	G
		H	17-18	2x					1	G
		L	17-18	2x					1	B
		I	17-20	3x					1	G
		K	17-18	3x					1	B
II	TCT	S							0	
		M	10-11	1x					1	P
		E	2	1x	11	1x	13	1x	3	P
		H	10-11	1x					1	P
		L	12-17	3x					1	G
		I	10-14	3x					1	G
		K	10-13	1x					1	P
III	TGH	S	30-34	1x					1	P
		M							0	
		E							0	
		H							0	
		L	17-19	2x	38-39	1x	41-43	1x	3	G
		I	38-40	2x	41-43	3x			2	G
		K	2-3	1x	9-11	1x			2	P
IV	TVB	S	11-12	1x					1	P
		M	11-12	1x					1	P
		E	10-11	1x	12-13	1x			2	P
		H	11-12	1x					1	P
		L	11-12	2x					1	G
		I							0	
		K	11-12	2x					1	G

* Refer page xvi for explanation of abbreviations

** Refer Table 2 for buffer systems

FIG. 22

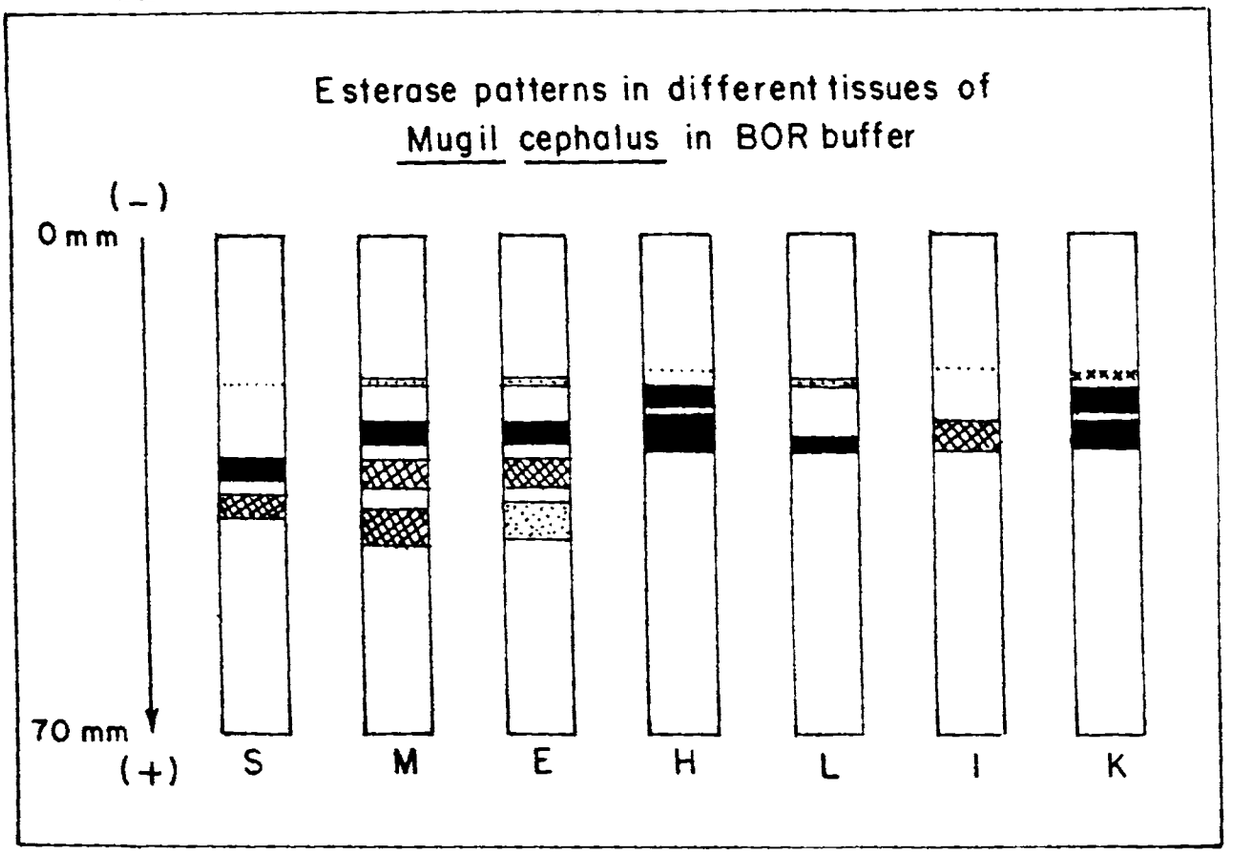

Esterase patterns in different tissues of
Mugil cephalus in BOR buffer
(-)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

FIG. 23

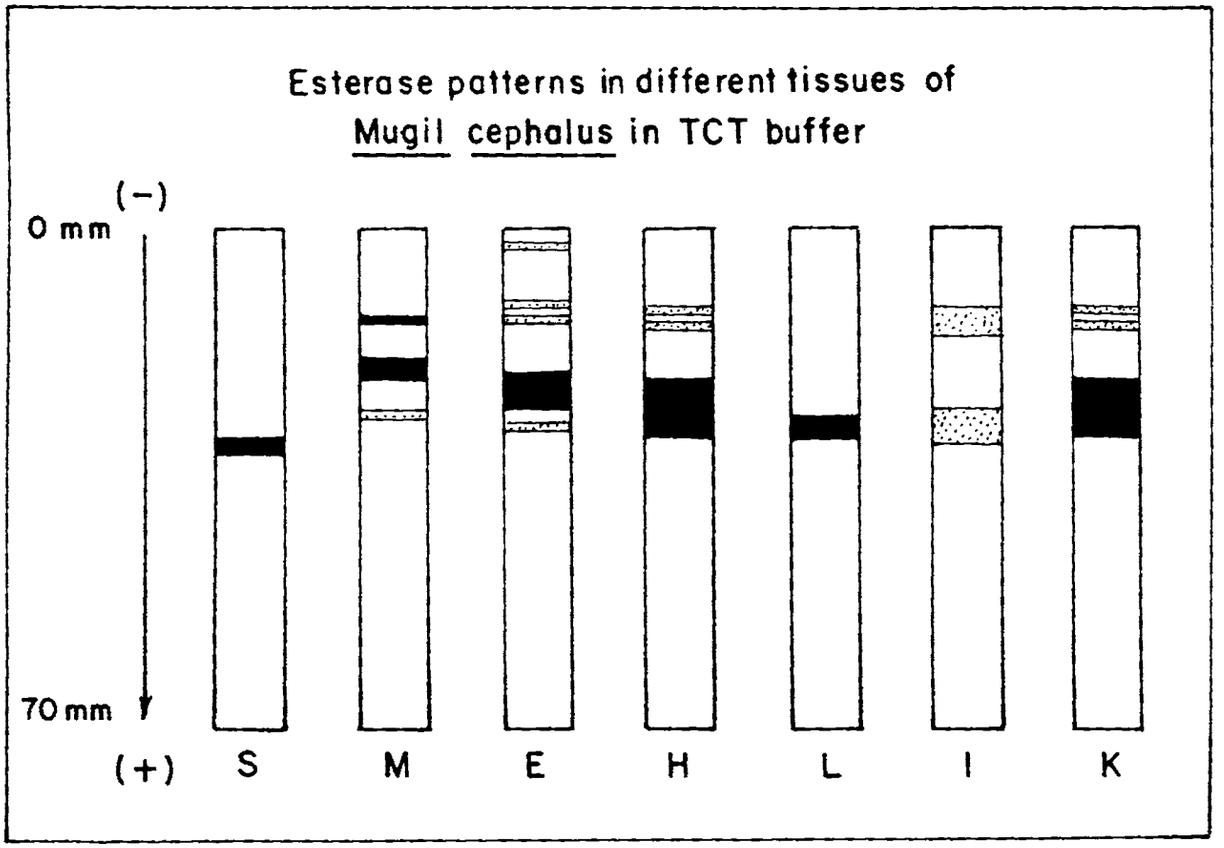

Esterase patterns in different tissues of
Mugil cephalus in TCT buffer
(-)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

FIG. 24

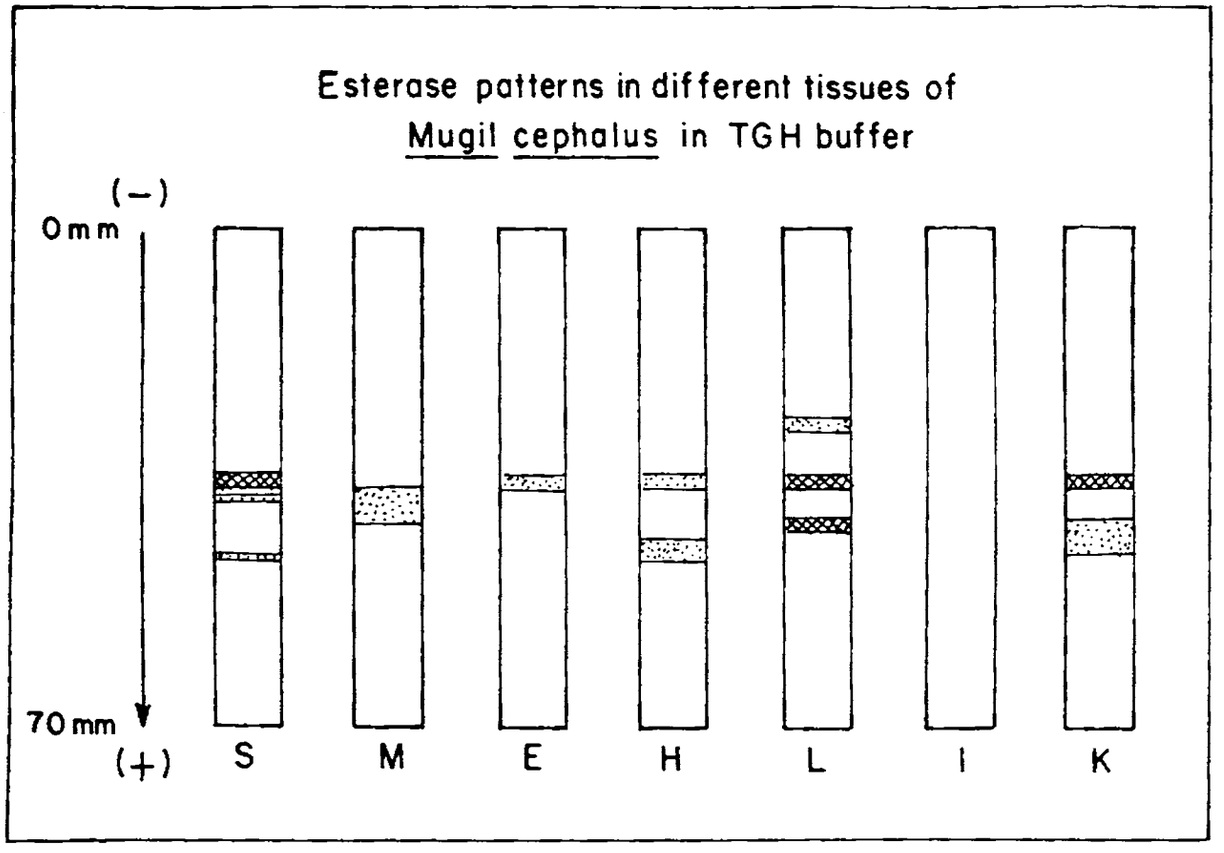

Esterase patterns in different tissues of
Mugil cephalus in TGH buffer
(−)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

FIG. 25

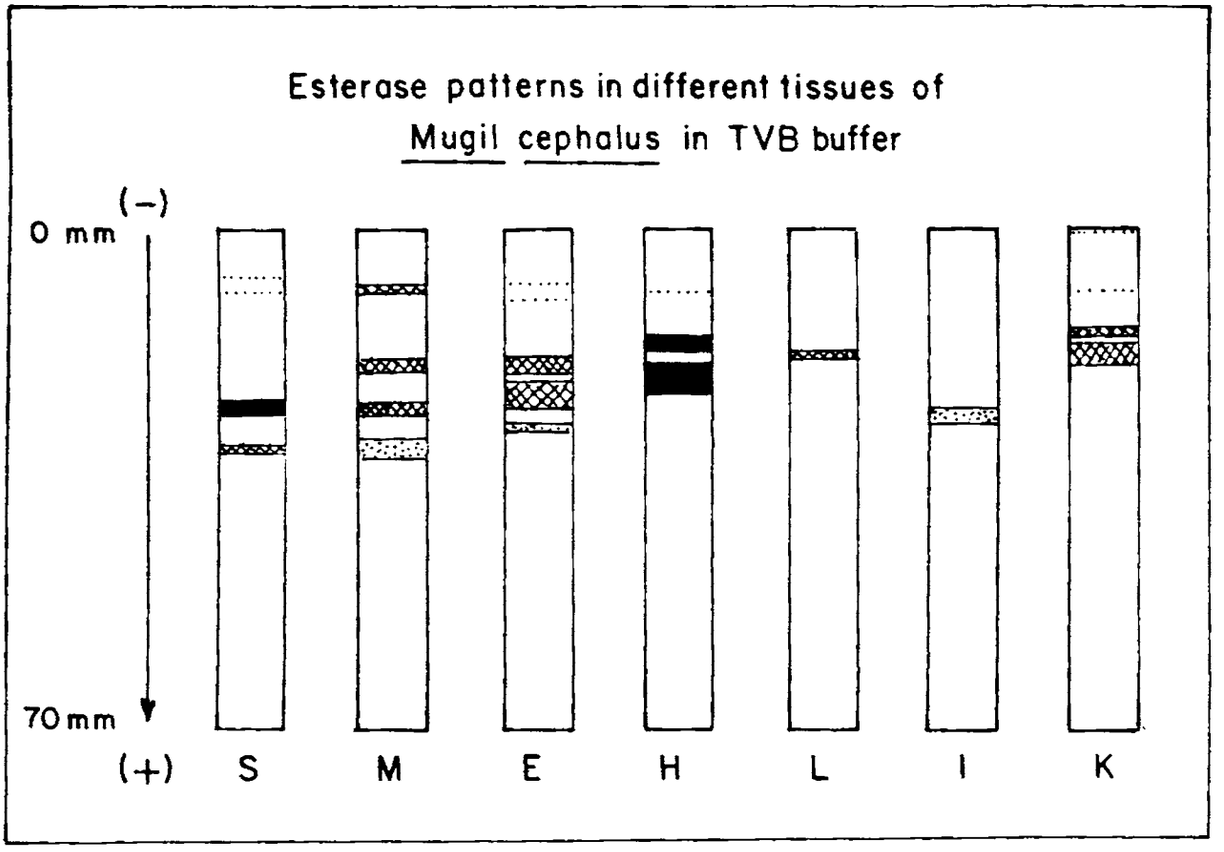

Esterase patterns in different tissues of
Mugil cephalus in TVB buffer
(−)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

PLATE 8

Esterase patterns in different tissues of *M. cephalus* in

A. Borate buffer

B. Tris Citrate buffer

C. Tris Glycine HCl buffer

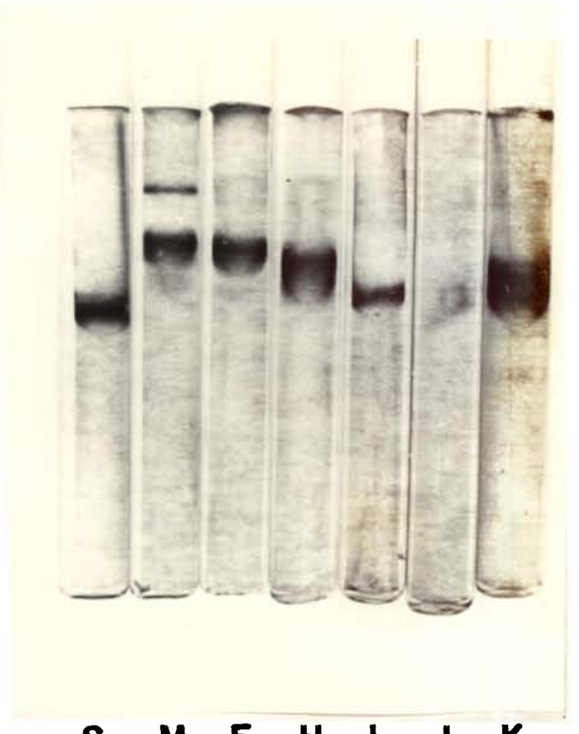
D. Tris Versene Borate buffer

PLATE 8

(A) EST-BOR

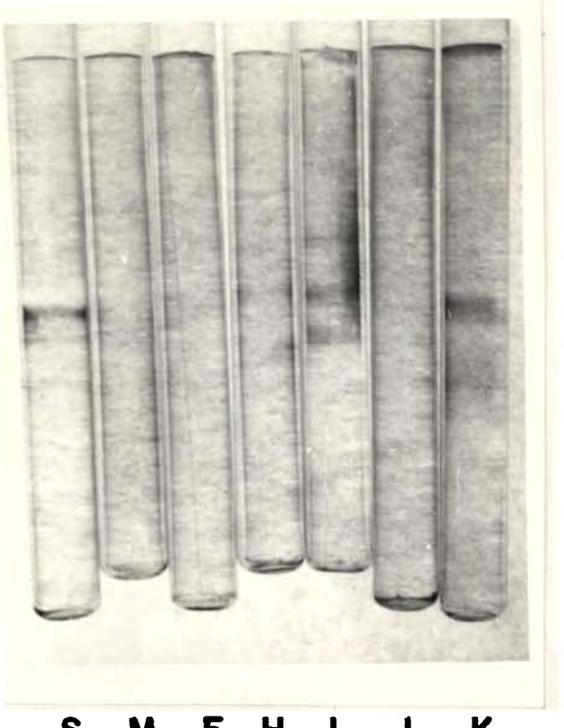
S M E H L I K

(B) EST-TCT



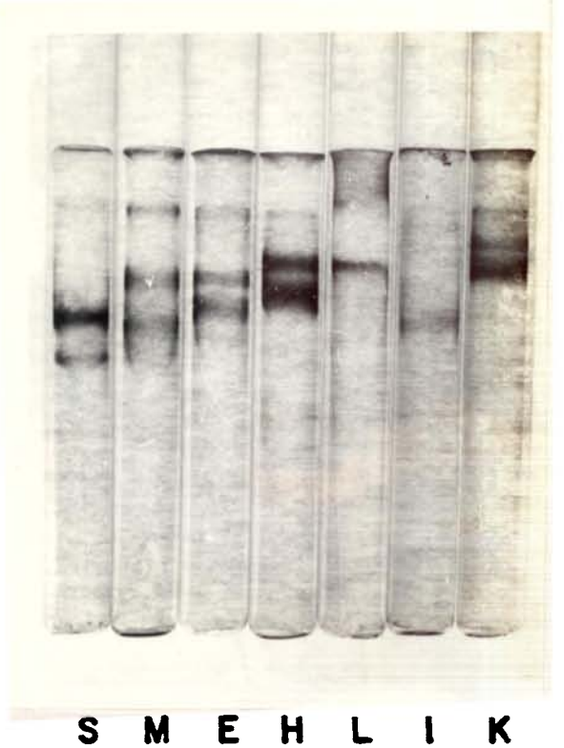
S M E H L I K

(C) EST-TGH



S M E H L I K

(D) EST-TVB



S M E H L I K

TABLE 9*

Experimental Details of Electrophoresis of ESTERASE in

Mugil cephalus L.

Sl. No.	Electrophoretic Buffer Systems **	Tissues	Relative Electrophoretic Mobility (mm) and Intensity (x) of Different Bands					Total No. of Bands	Nature of Separation and Resolution
			1	2	3	4	5		
I	BOR	S	21 1x	31-34 3x	36-39 2x			3	G
		M	20-21 1x	26-29 3x	31-35 2x	38-43 2x		4	G
		E	20-21 1x	26-29 3x	31-35 2x	37-42 1x		4	P
		H	19 1x	21-24 3x	25-30 3x			3	G
		L	20-21 1x	28-30 3x				2	G
		I	19 1x	26-30 2x				2	P
		K	20 2x	22-25 3x	26-30 3x			3	G
II	TCT	S	29-31 3x					1	G
		M	12-13 3x	18-21 3x	25-26 1x			3	G
		E	2-3 1x	10-11 1x	12-13 1x	20-25 3x	27-28 1x	5	P
		H	11-12 1x	13-14 1x	21-29 3x			3	P
		L	26-29 3x					1	G
		I	11-15 1x	25-30 1x				2	P
		K	11-12 1x	13-14 1x	21-29 3x			3	P
III	TGH	S	34-36 2x	37-38 1x	45-46 1x			3	P
		M	36-41 1x					1	P
		E	34-36 1x					1	P
		H	34-36 1x	43-46 1x				2	P
		L	26-28 1x	34-36 2x	40-42 2x			3	G
		I						0	
		K	34-36 2x	42-45 1x				2	P
IV	TVB	S	7 1x	9 1x	24-26 3x	30-31 2x		4	G
		M	8-9 2x	18-20 2x	24-26 2x	29-32 1x		4	G
		E	8 1x	10 1x	18-20 2x	21-25 2x	27-28 1x	5	G
		H	9 1x	15-17 3x	19-23 3x			3	B
		L	17-18 2x					1	G
		I	25-27 1x					1	P
		K	1 1x	9 1x	14-15 2x	16-19 2x		4	G

* Refer Page xvi for explanation of abbreviations

** Refer Table 2 for buffer systems

FIG. 26

Glutamate Dehydrogenase patterns in different tissues of Mugil cephalus in SCT buffer

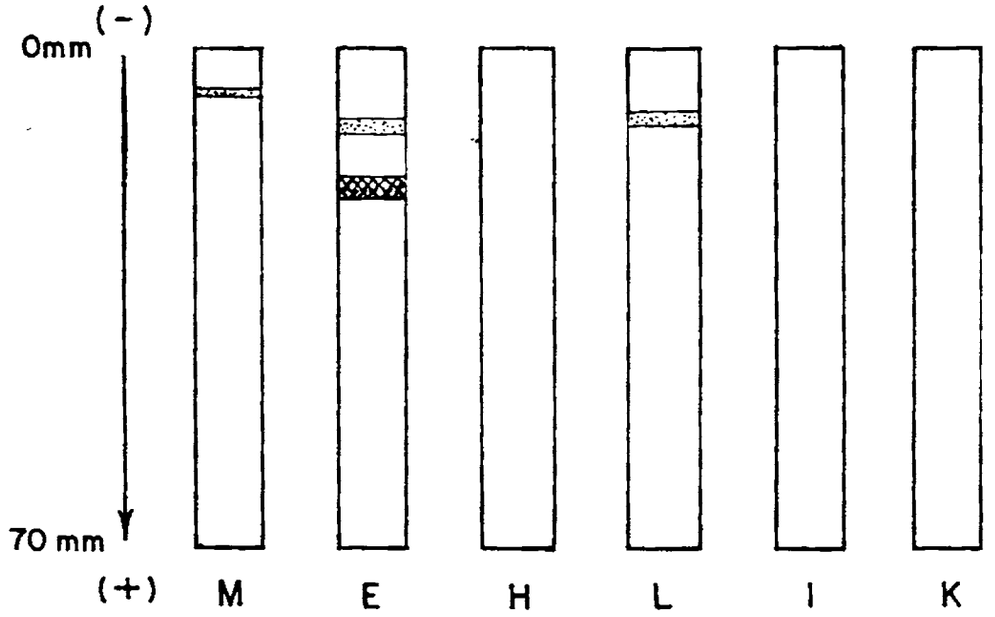


FIG. 27

Glutamate Dehydrogenase patterns in different tissues of Mugil cephalus in TCT buffer

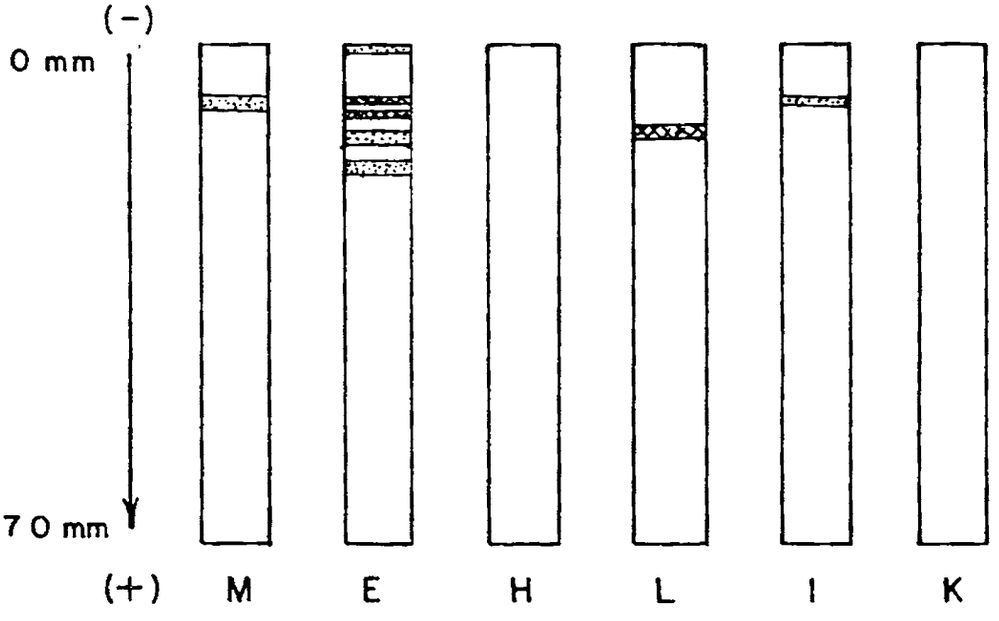


FIG. 28

Glutamate Dehydrogenase patterns in different tissues of Mugil cephalus in TME buffer

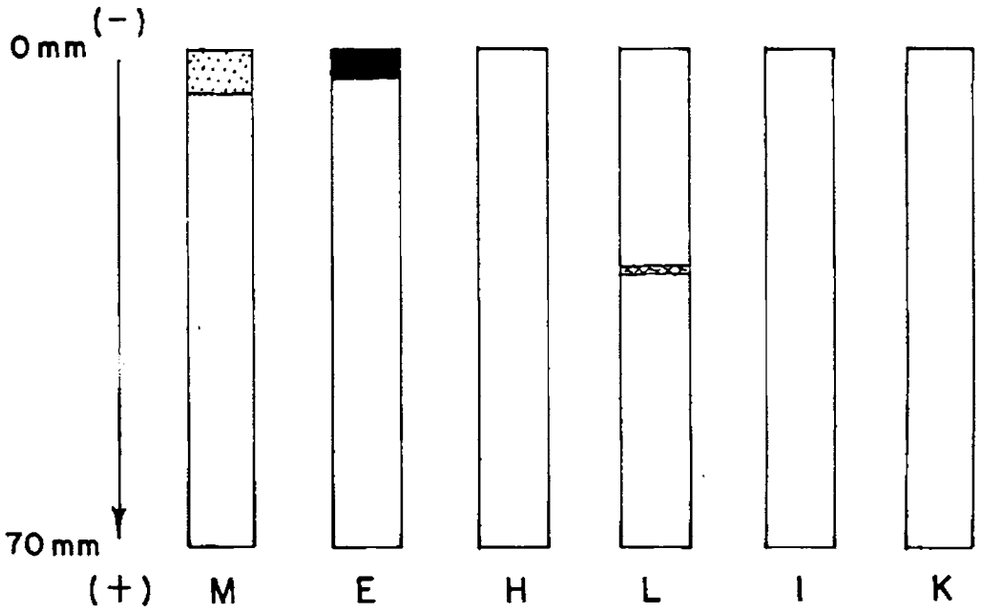


FIG. 29

Glutamate Dehydrogenase patterns in different tissues of Mugil cephalus in TVB buffer

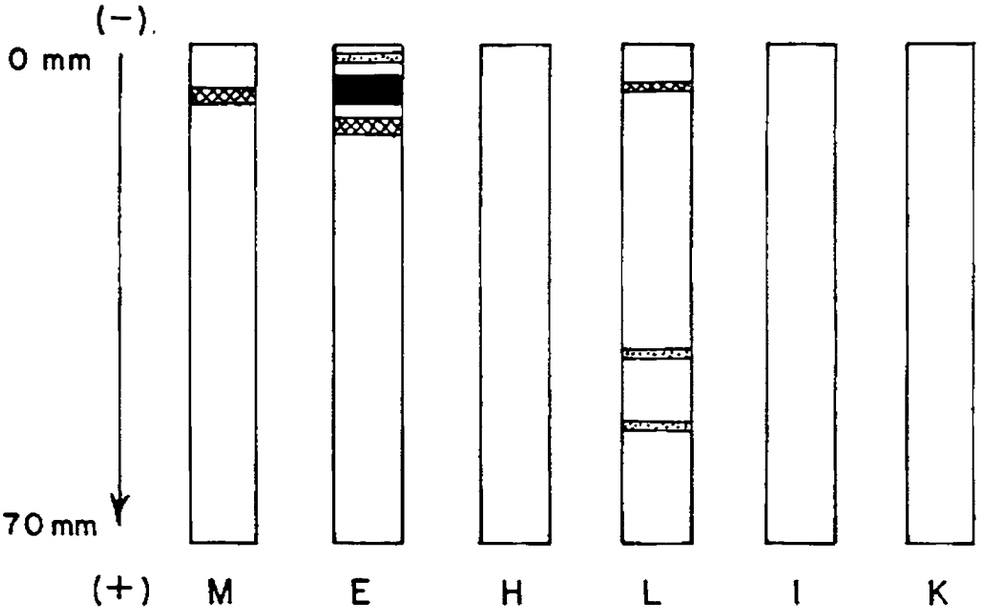


PLATE 9

Glutamate Dehydrogenase patterns in different tissues of M. cephalus in

A. Sodium Citrate buffer

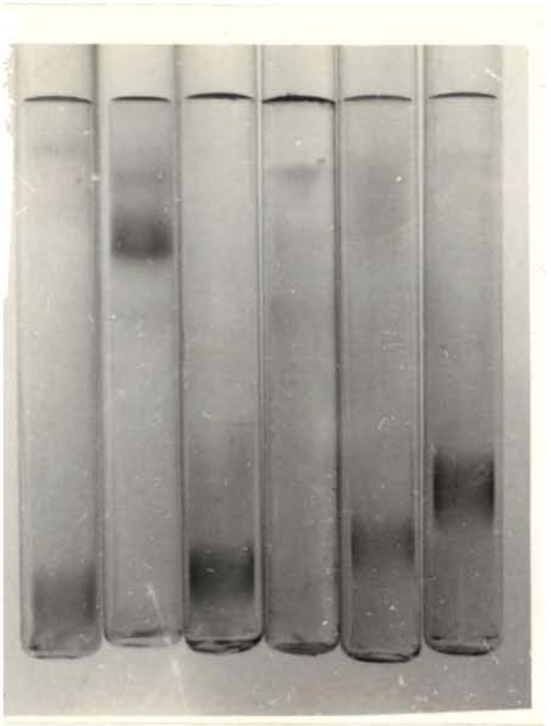
B. Tris Citrate buffer

C. Tris Maleic EDTA buffer

D. Tris Versene Borate buffer

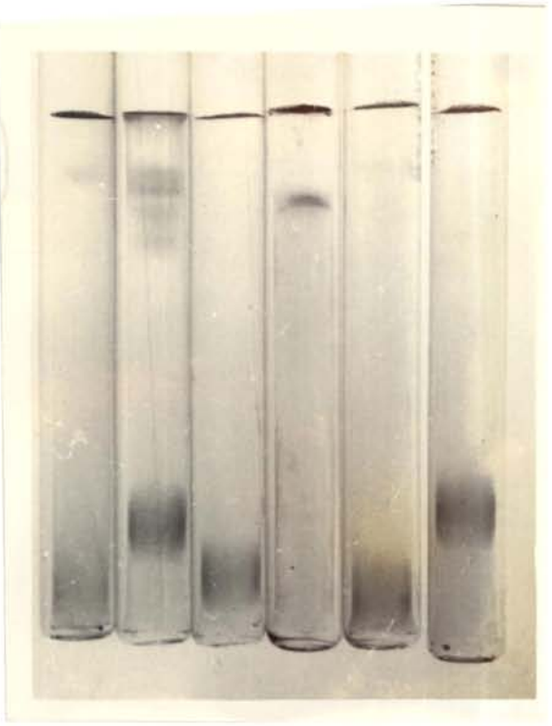
PLATE 9

(A) GDH-SCT



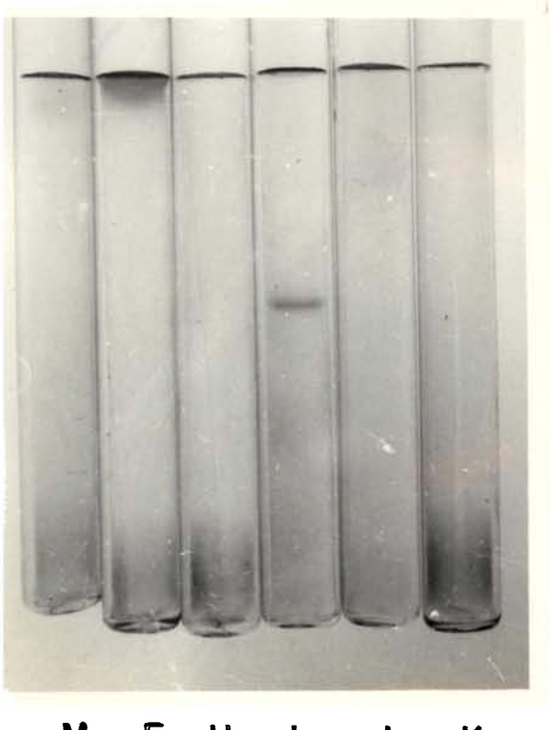
M E H L I K

(B) GDH-TCT



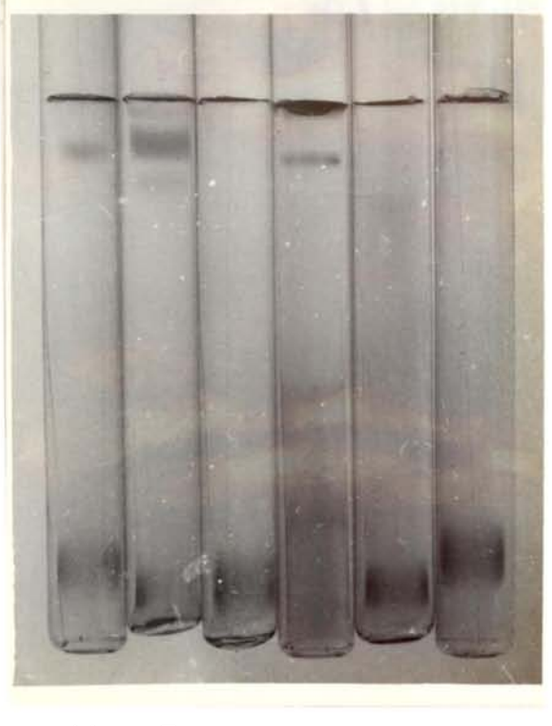
M E H L I K

(C) GDH-TME



M E H L I K

(D) GDH-TVB



M E H L I K

TABLE 10*

Experimental Details of Electrophoresis of GLUTAMATE DEHYDROGENASE in *Mugil cephalus* L.

Sl. No.	Electrophoretic Buffer Systems **	Tissues	Relative Electrophoretic Mobility (mm) Intensity (x) of Different Bands										Total No. of Bands	Nature of Separation and Resolution
			1		2		3		4		5			
I	SCT	M	6-7	1x									1	P
		E	10-12	1x	18-21	2x							2	P
		H											0	
		L	9-11	1x									1	P
		I											0	
		K											0	
II	TCT	M	7-9	1x									1	P
		E	0-1	1x	7-8	2x	9-10	2x	12-14	1x	16-18	1x	5	B
		H											0	
		L	11-13	2x									1	G
		I	7-8	1x									1	P
		K											0	
III	TME	M	0-6	1x									1	P
		E	0-4	3x									1	G
		H											0	
		L	30-31	2x									1	G
		I											0	
		K											0	
IV	TVB	M	6-8	2x									1	G
		E	1-2	1x	4-8	3x	10-12	2x					3	G
		H											0	
		L	5-6	2x	42-43	1x	52-53	1x					3	G
		I											0	
		K											0	

* Refer Page xvi for explanation of abbreviations

** Refer Table 2 for buffer systems

FIG.30

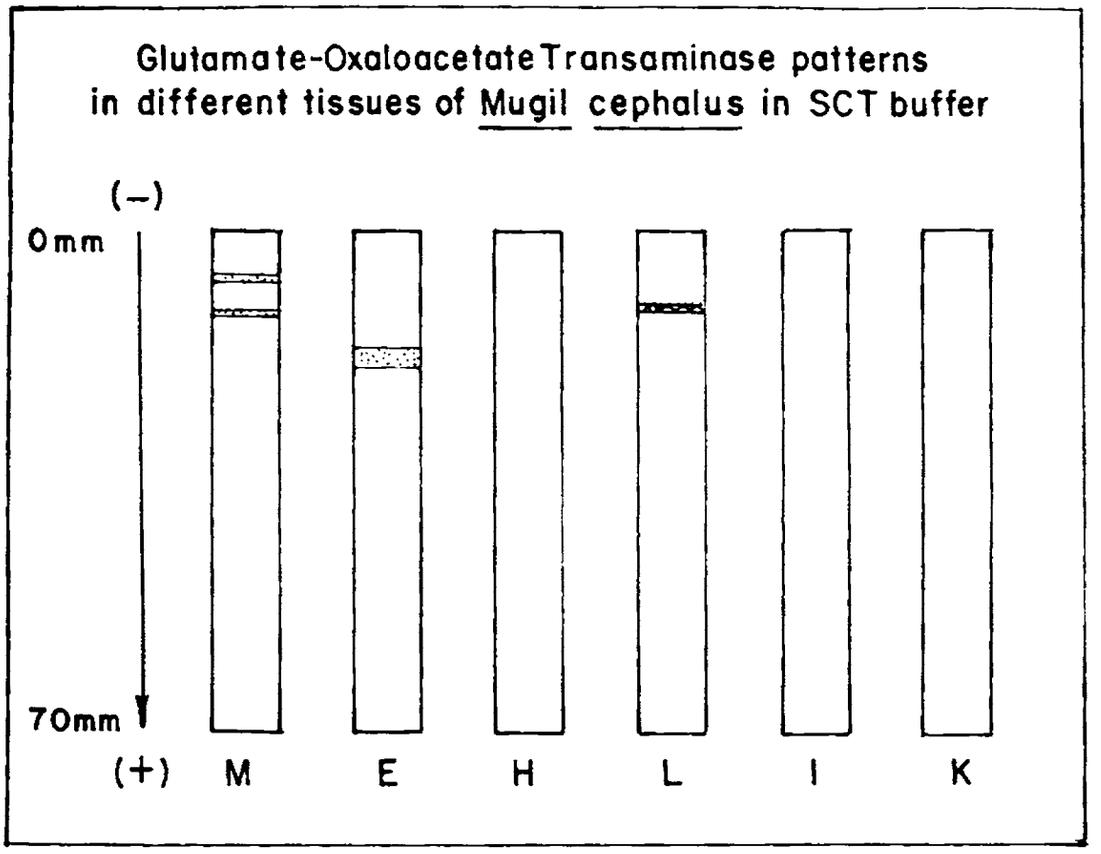

Glutamate-Oxaloacetate Transaminase patterns
in different tissues of Mugil cephalus in SCT buffer
(–)
0mm
70mm
(+)
M
E
H
L
I
K

FIG.31

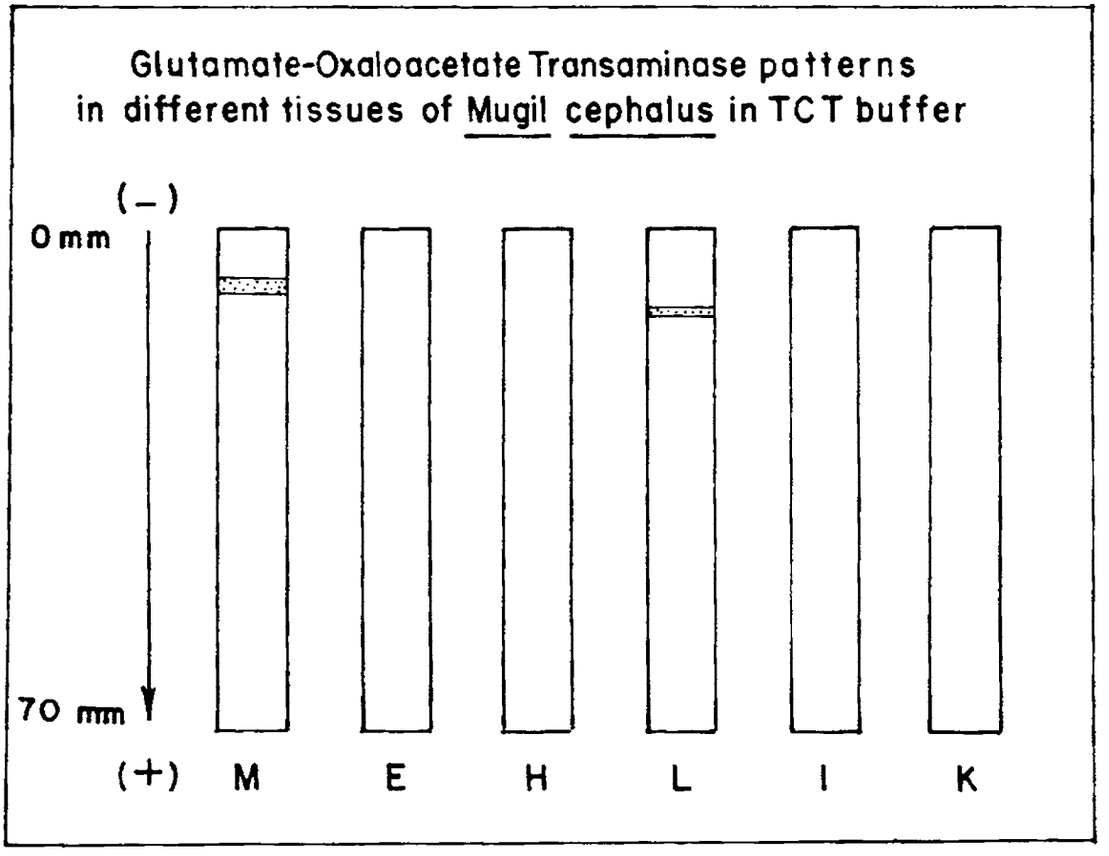

Glutamate-Oxaloacetate Transaminase patterns
in different tissues of Mugil cephalus in TCT buffer
(–)
0mm
70 mm
(+)
M
E
H
L
I
K

FIG. 32

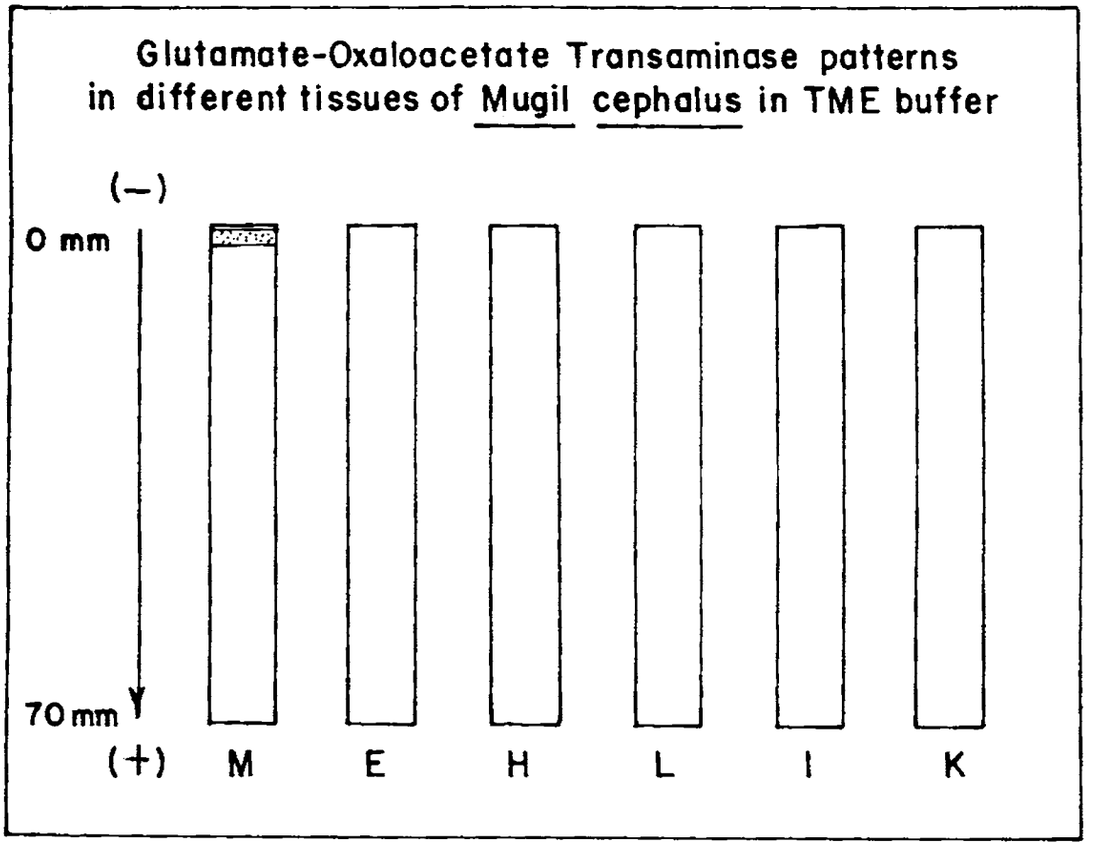

Glutamate-Oxaloacetate Transaminase patterns
in different tissues of Mugil cephalus in TME buffer
(−)
0 mm
70 mm
(+)
M
E
H
L
I
K

FIG. 33

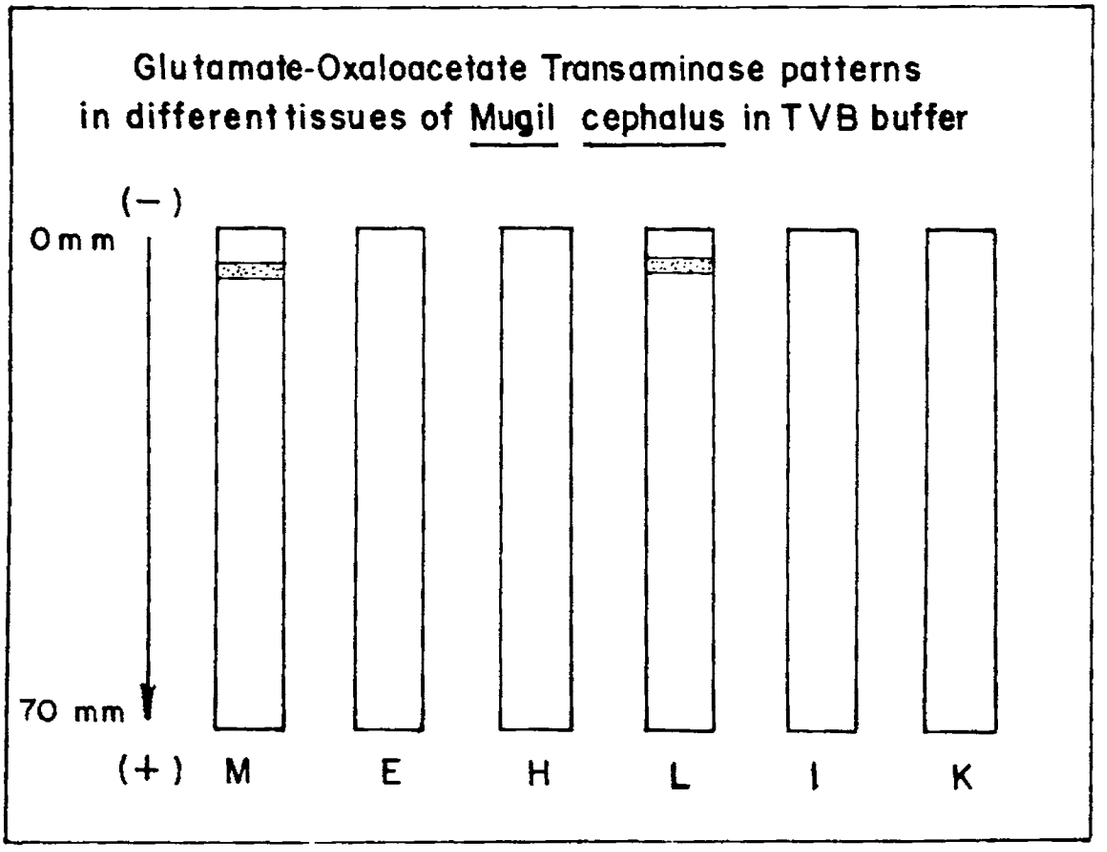

Glutamate-Oxaloacetate Transaminase patterns
in different tissues of Mugil cephalus in TVB buffer
(−)
0 mm
70 mm
(+)
M
E
H
L
I
K

PLATE 10

Glutamate-Oxaloacetate Transaminase patterns of different tissues of M. cephalus in

A. Sodium Citrate buffer

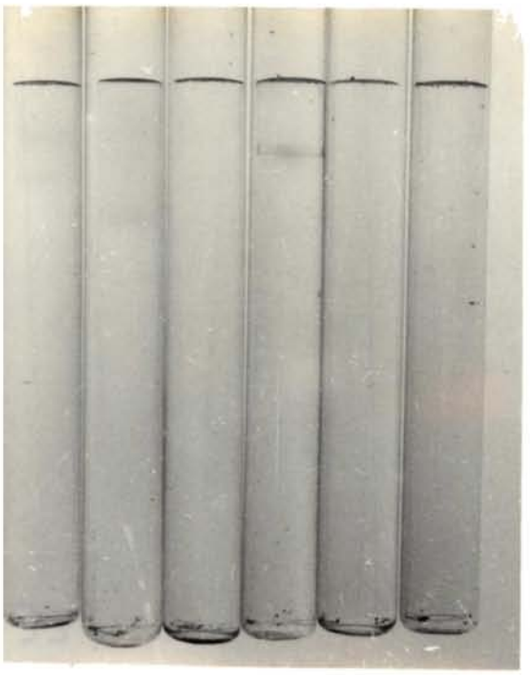
B. Tris Citrate buffer

C. Tris Maleic EDTA buffer

D. Tris Versene Borate buffer

PLATE 10

(A) GOT-SCT

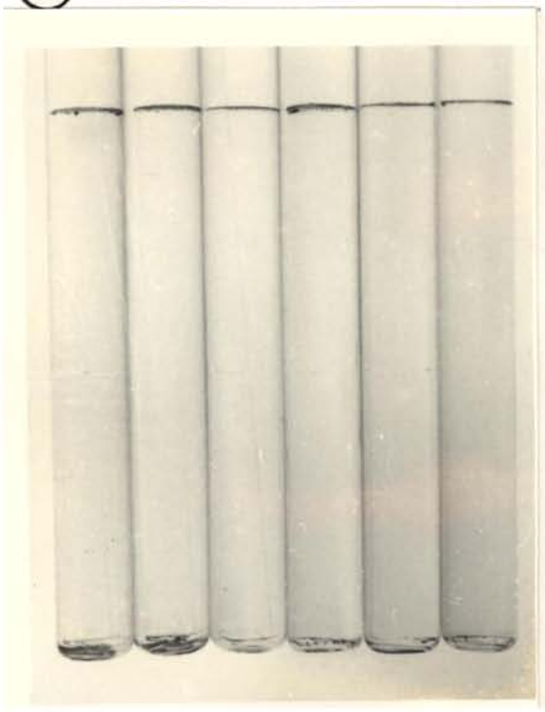


M E H L I K

(B) GOT-TCT

M E H L I K

(C) GOT-TME



(D) GOT-TVB

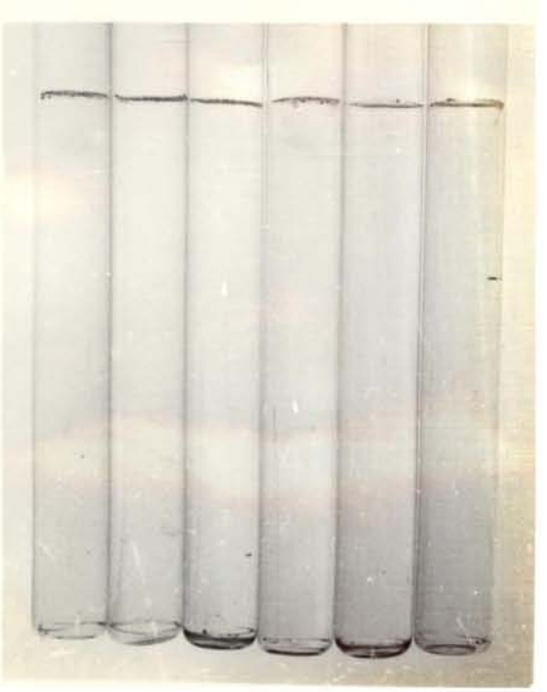


TABLE 11*

Experimental Details of Electrophoresis of GLUTAMATE - OXALOACETATE TRANSAMINASE in *Mugil cephalus* L.

Sl. No.	Electrophoretic Buffer Systems **	Tissues	Relative Electrophoretic Mobility (mm) and Intensity (x) of Different Bands		Total No. of Bands	Nature of Separation and Resolution
			1	2		
I	SCT	M	6-7 1x	11-12 1x	2	B
		E	16-19 1x		1	G
		H			0	
		L	10-11 2x		1	G
		I			0	
		K			0	
II	TCT	M	7-9 1x		1	P
		E			0	
		H			0	
		L	11-12 1x		1	P
		I			0	
		K			0	
III	TME	M	1-3 1x		1	P
		E			0	
		H			0	
		L			0	
		I			0	
		K			0	
IV	TVB	M	5-7 1x		1	P
		E			0	
		H			0	
		L	4-6 1x		1	P
		I			0	
		K			0	

* Refer Page xvi for exaplanation of abbreviations

** Refer Table 2 for buffer systems

FIG. 34

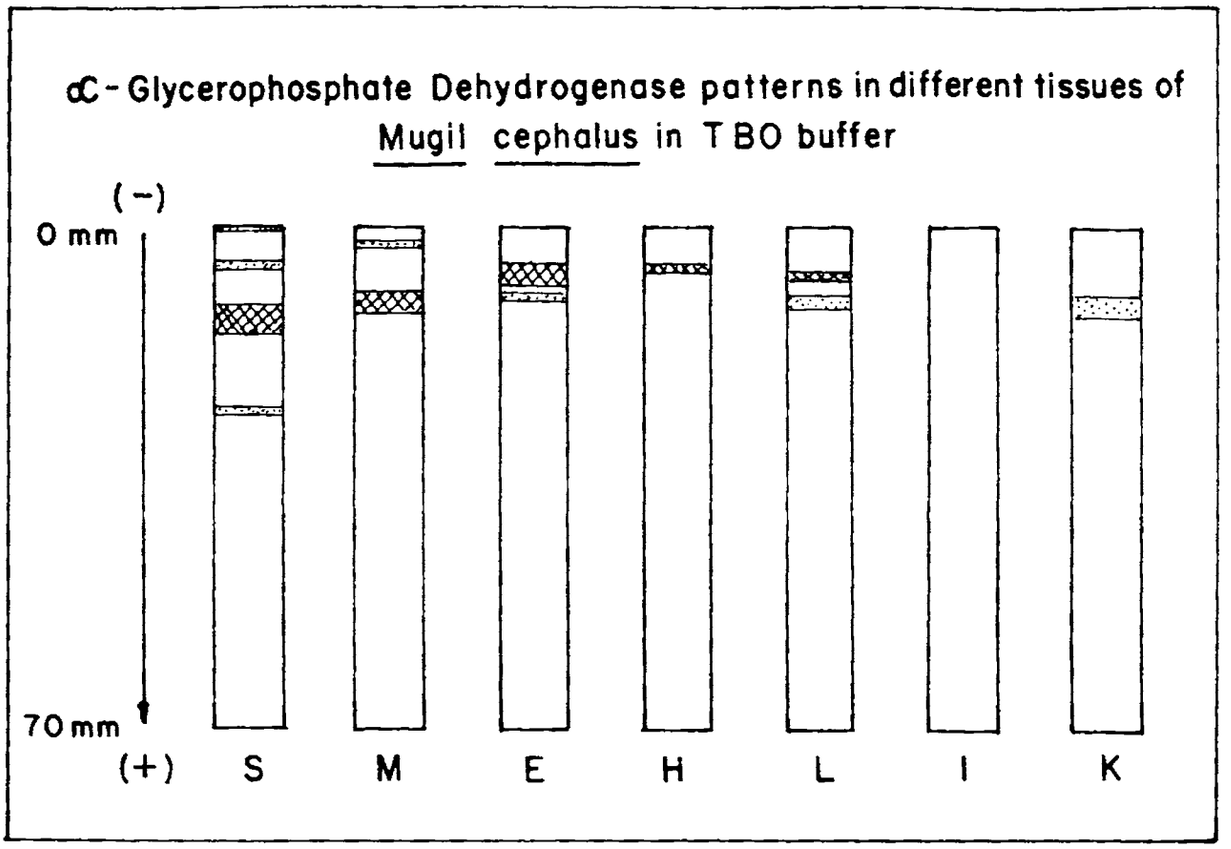

αC - Glycerophosphate Dehydrogenase patterns in different tissues of Mugil cephalus in TBO buffer
(-)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

FIG. 35

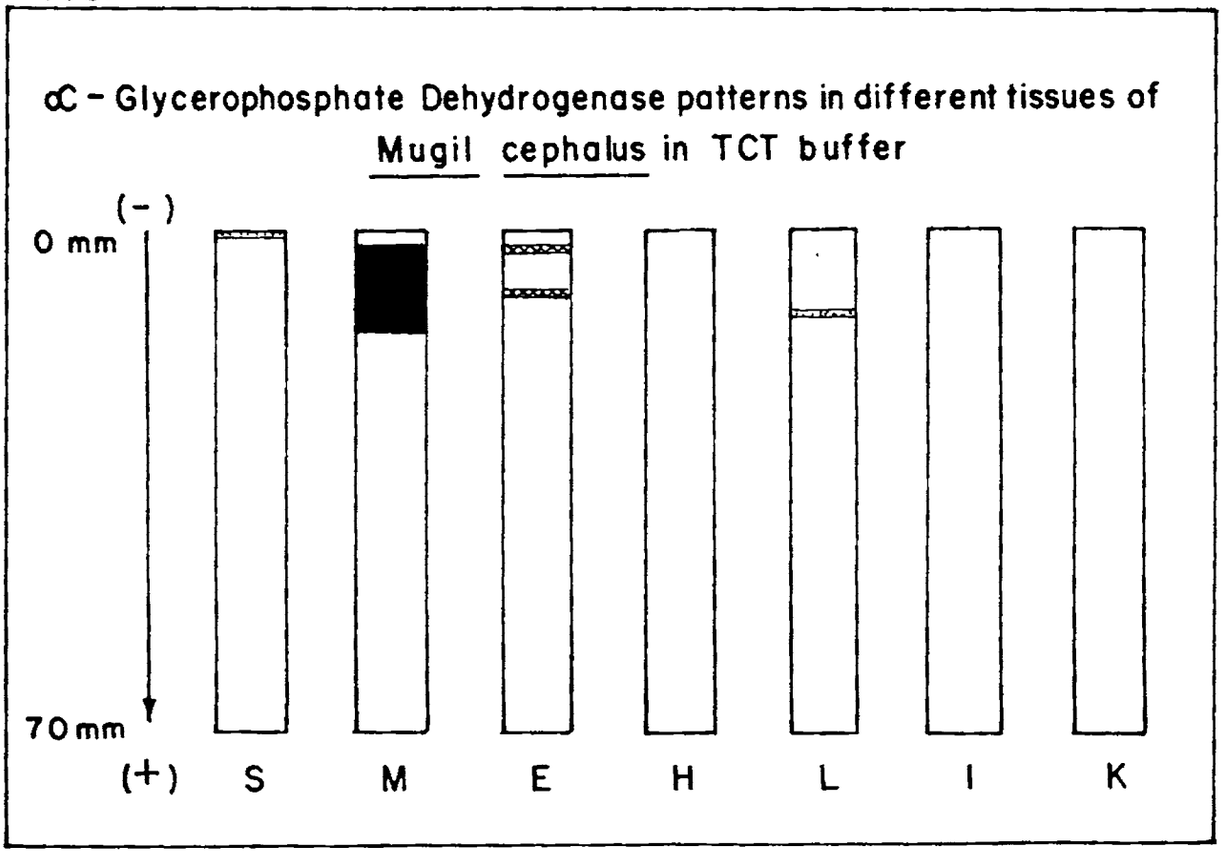

αC - Glycerophosphate Dehydrogenase patterns in different tissues of Mugil cephalus in TCT buffer
(-)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

FIG. 36

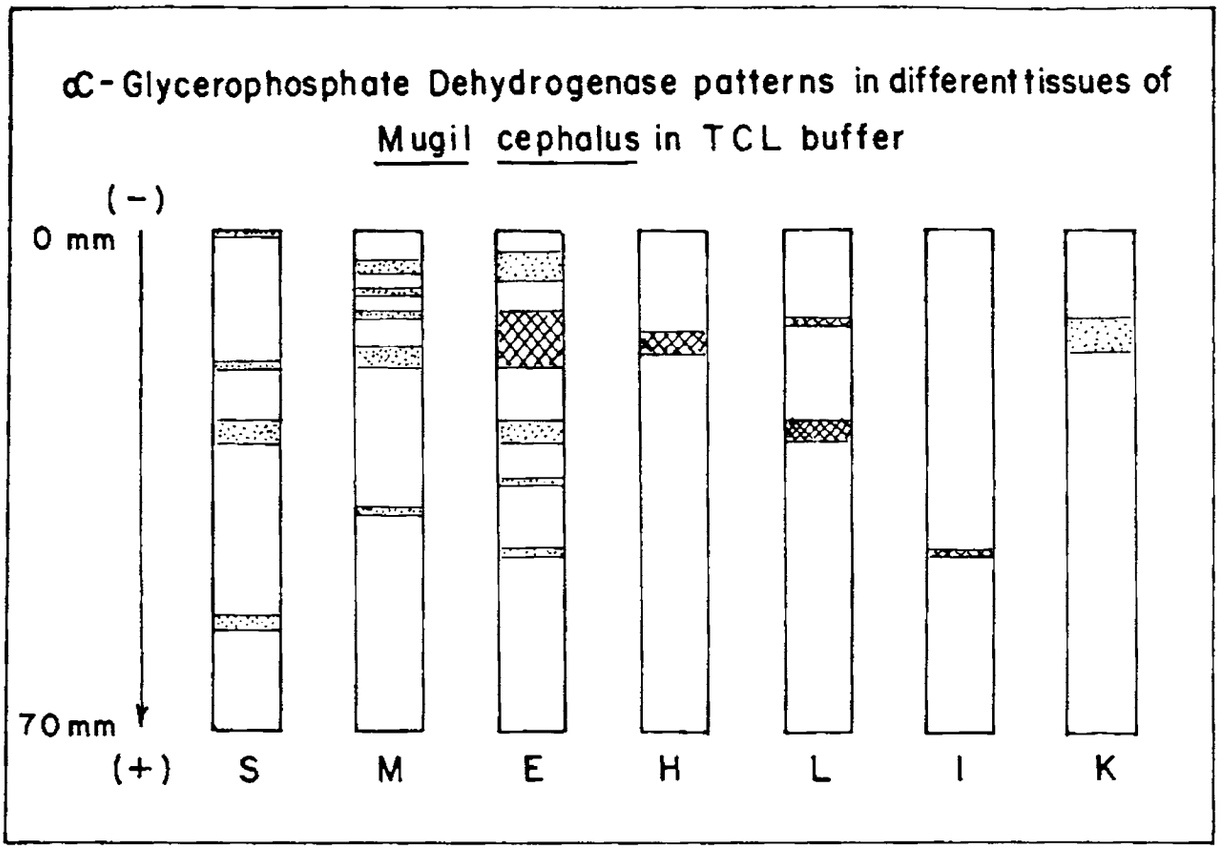

αC - Glycerophosphate Dehydrogenase patterns in different tissues of
Mugil cephalus in TCL buffer
(-)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

FIG. 37

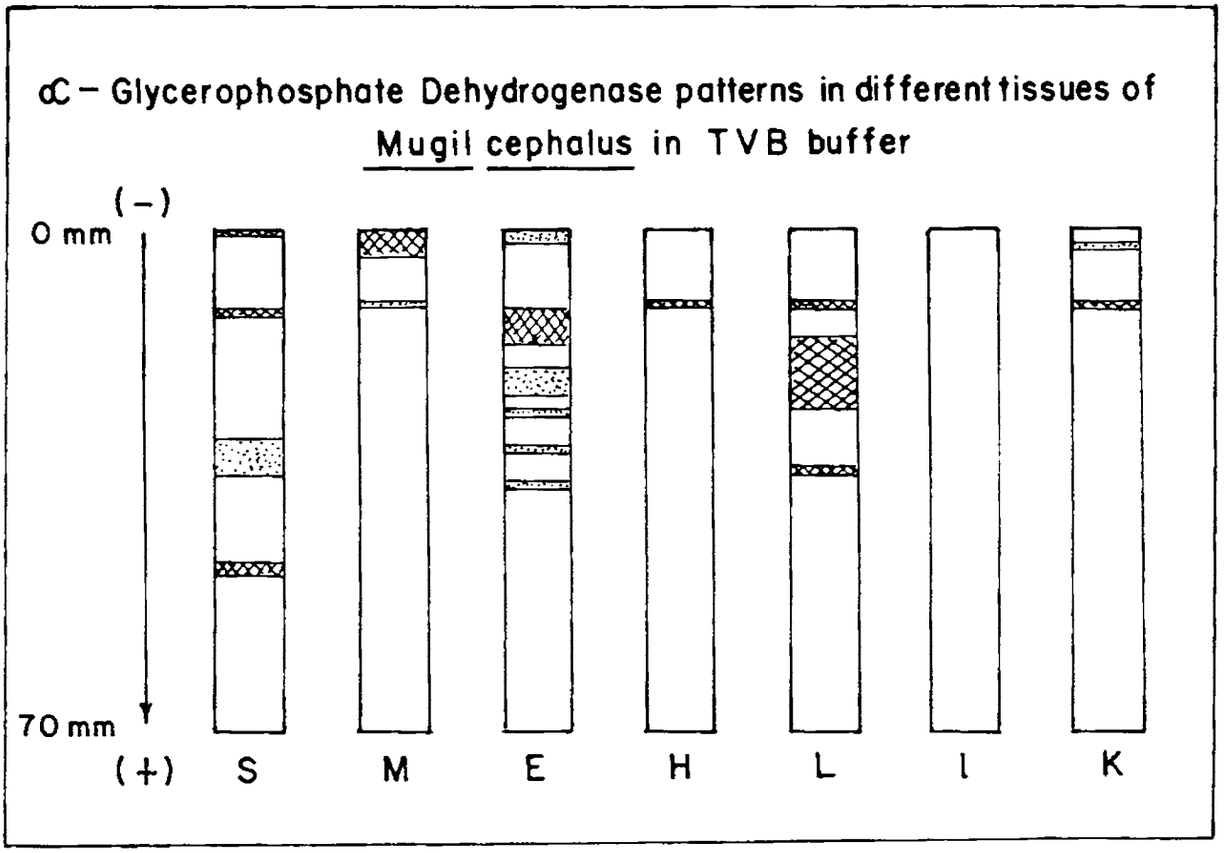

αC - Glycerophosphate Dehydrogenase patterns in different tissues of
Mugil cephalus in TVB buffer
(-)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

TABLE 12* Experimental Details of Electrophoresis of ALPHA-GLYCEROPHOSPHATE DEHYDROGENASE in *Mugil cephalus* L.

Sl. No.	Electrophoretic Buffer Systems **	Tissues	Relative Electrophoretic Mobility (mm) and Intensity (x) of Different Bands						Total No. of Bands	Nature of Separation and Resolution
			1	2	3	4	5	6		
I	TBO	S	0-1 1x	5-6 1x	11-15 2x	25-26 1x			4	P
		M	2-3 1x	9-12 2x					2	G
		E	5-8 2x	9-10 1x					2	G
		H	5-6 2x						1	G
		L	6-7 2x	9-11 1x					2	G
		I							0	
		K	9-12 1x						1	P
II	TCT	S	0-1 1x						1	P
		M	2-14 3x						1	P
		E	2-3 2x	8-9 2x					2	G
		H							0	
		L	11-12 1x						1	P
		I							0	
		K							0	
III	TCL	S	0-1 1x	18-19 1x	26-29 1x	53-55 1x			4	P
		M	4-6 1x	8-9 1x	11-12 1x	16-19 1x	38-39 1x		5	P
		E	3-7 1x	11-19 2x	26-29 1x	34-35 1x	44-45 1x		5	P
		H	14-17 2x						1	G
		L	12-13 2x	26-29 2x					2	G
		I	44-45 2x						1	G
		K	12-17 1x						1	P
IV	TVB	S	0-1 2x	11-12 2x	29-34 1x	46-48 2x			4	G
		M	0-4 2x	10-11 1x					2	G
		E	0-2 1x	11-16 2x	19-23 1x	25-26 1x	30-31 1x	35-36 1x	6	P
		H	10-11 2x						1	G
		L	10-11 2x	15-25 2x	33-34 2x				3	B
		I							0	
		K	2-3 1x	10-11 2x					2	G

* Refer Page xvi for explanation of abbreviations

** Refer Table 2 for buffer systems

FIG. 38

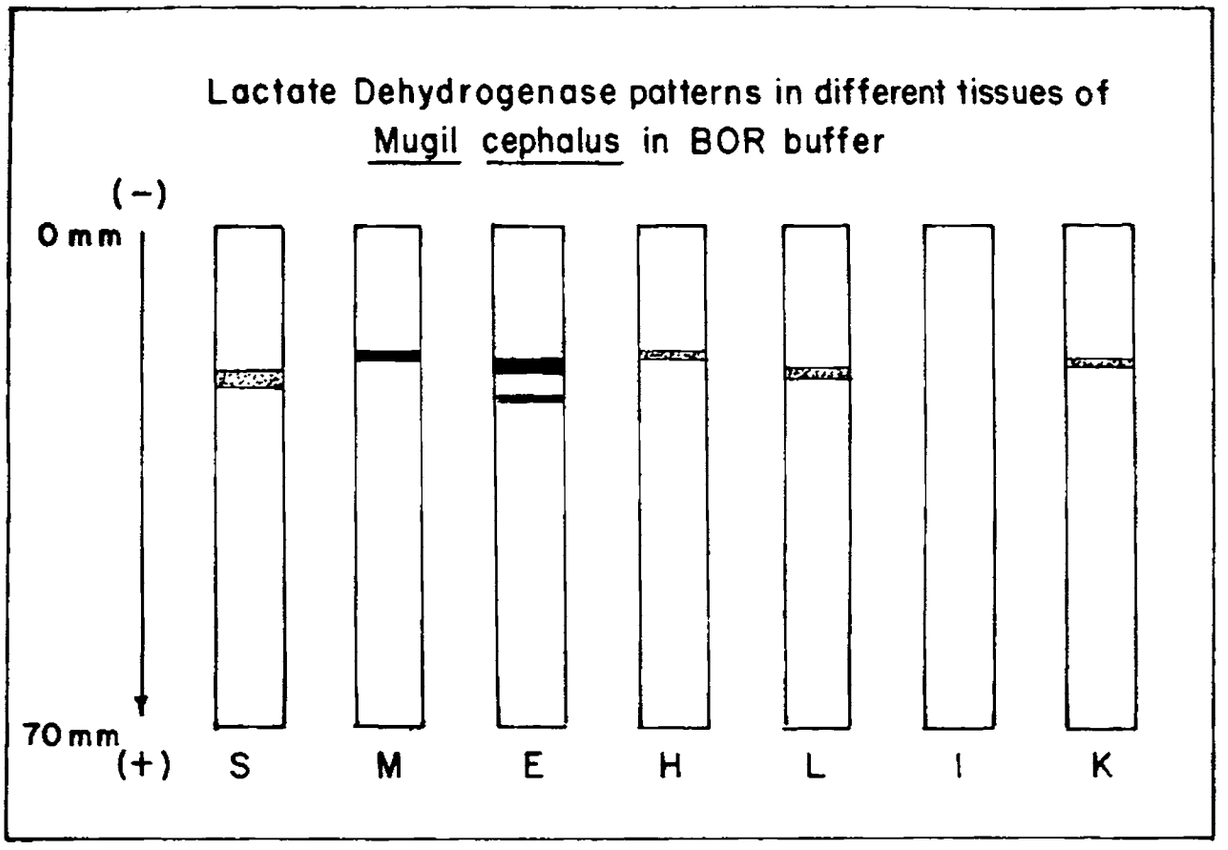

Lactate Dehydrogenase patterns in different tissues of
Mugil cephalus in BOR buffer
(-)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

FIG. 39

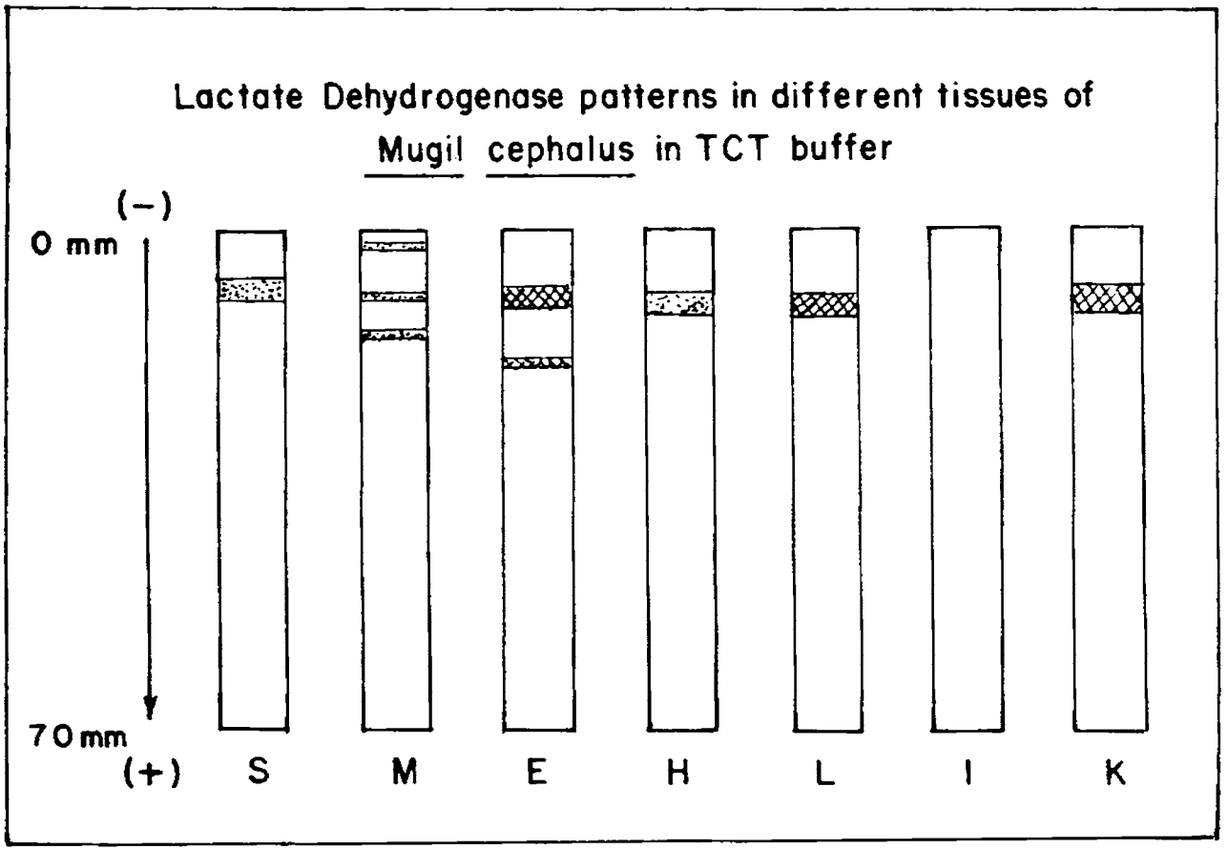

Lactate Dehydrogenase patterns in different tissues of
Mugil cephalus in TCT buffer
(-)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

FIG. 40

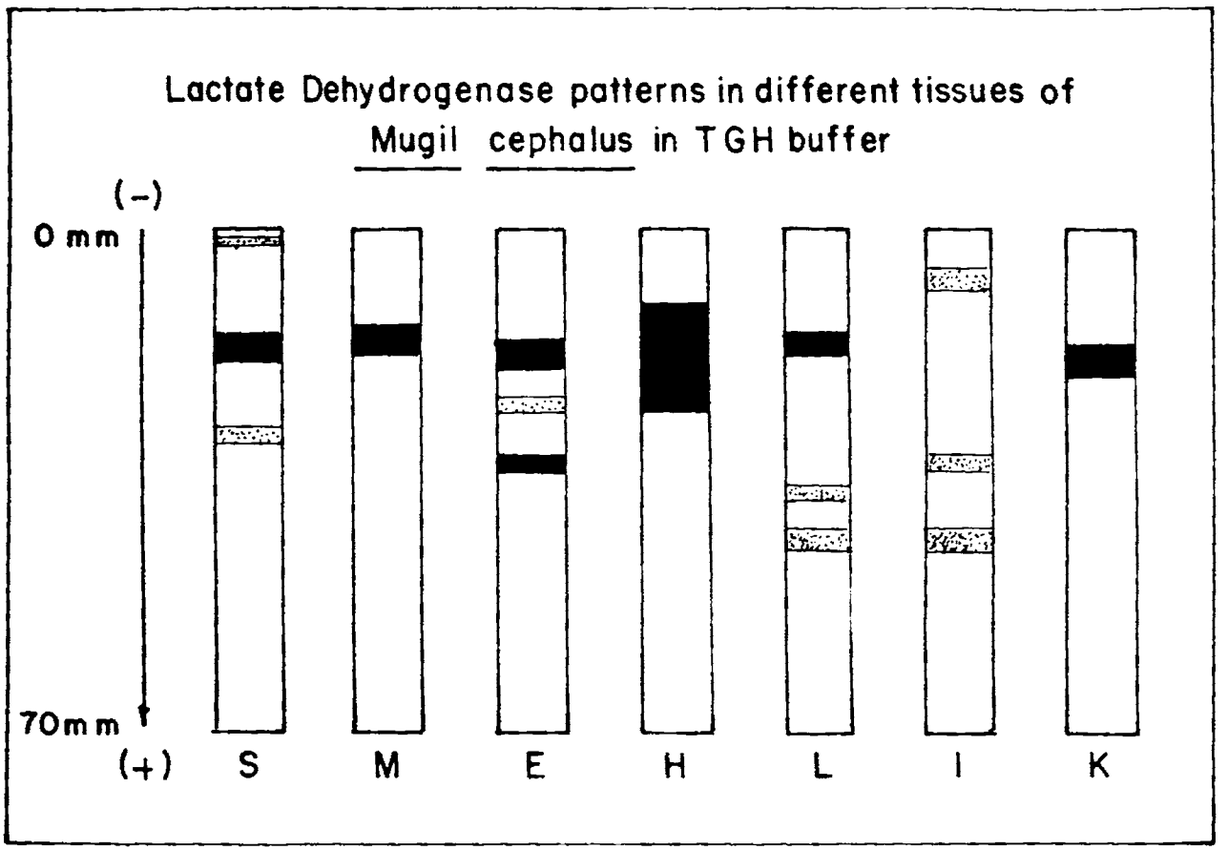

Lactate Dehydrogenase patterns in different tissues of
Mugil cephalus in TGH buffer
(-)
0 mm
70mm
(+)
S
M
E
H
L
I
K

FIG. 41

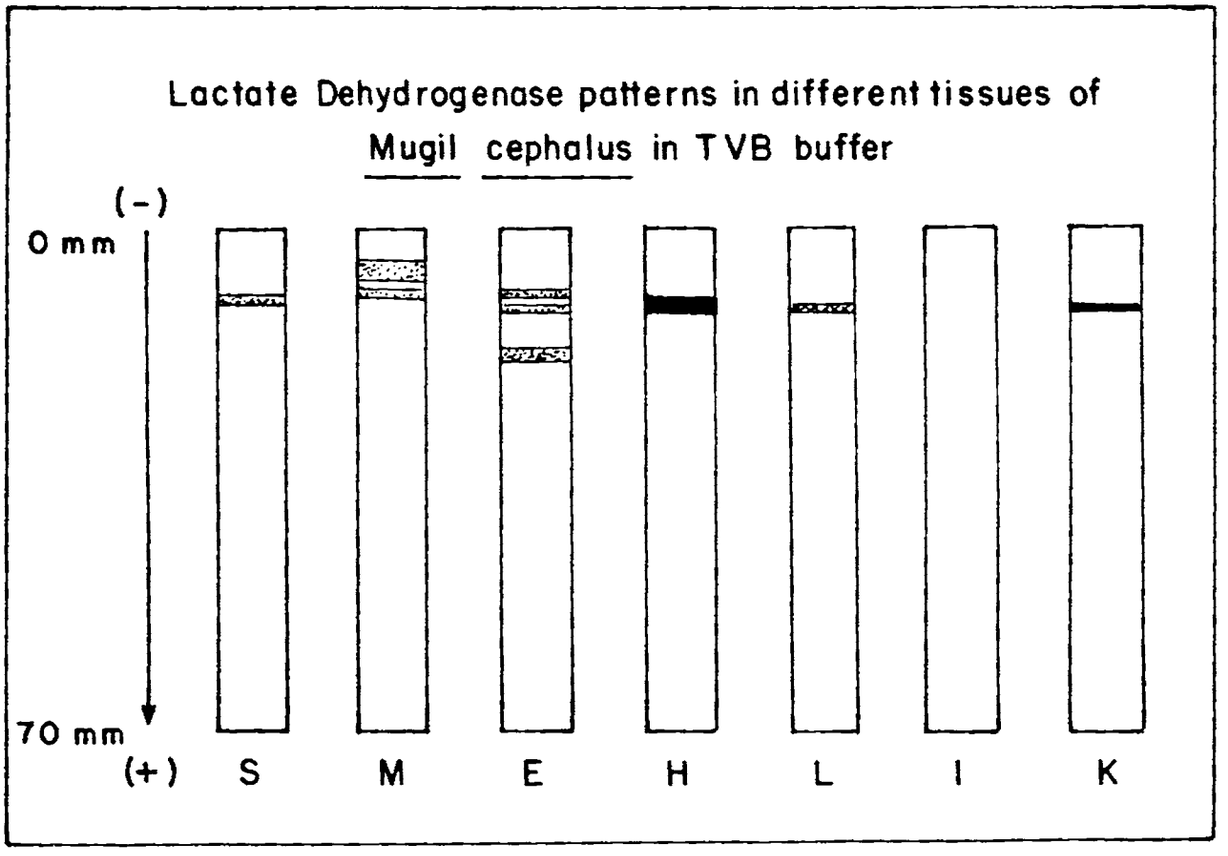

Lactate Dehydrogenase patterns in different tissues of
Mugil cephalus in TVB buffer
(-)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

PLATE 11

Lactate Dehydrogenase patterns in different tissues of M. cephalus in

A. Borate buffer

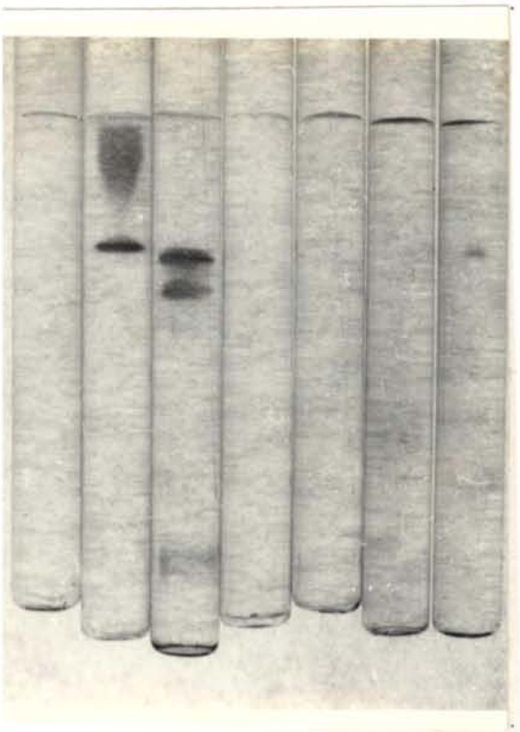
B. Tris Citrate buffer

C. Tris Glycine HCl buffer

D. Tris Versene Borate buffer

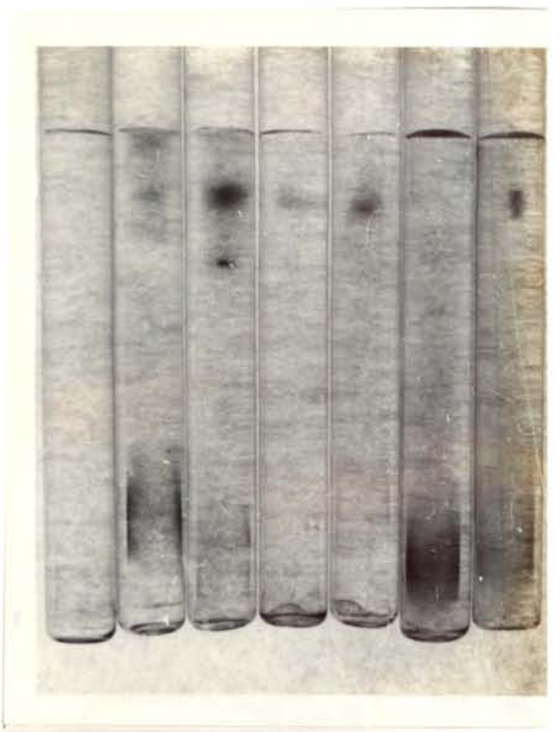
PLATE 11

(A) LDH-BOR



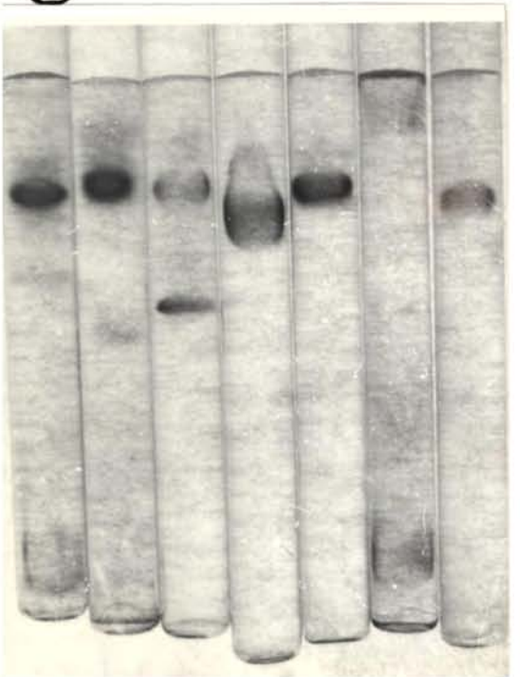
S M E H L I K

(B) LDH-TCT



S M E H L I K

(C) LDH-TGH



(D) LDH-TVB

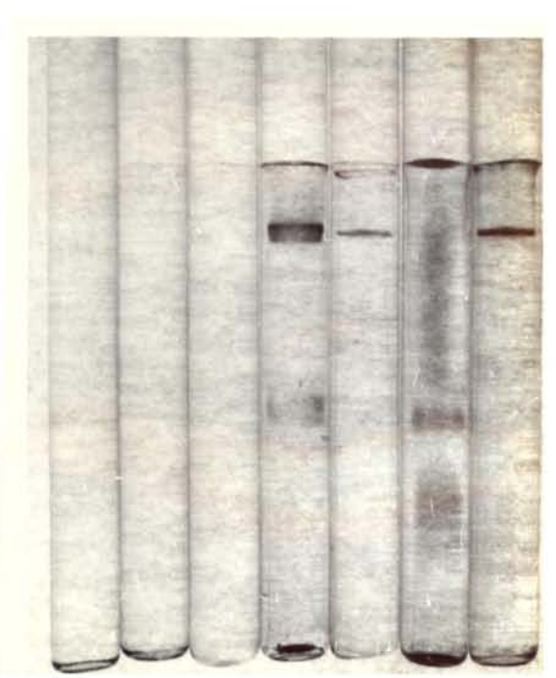


TABLE 13*

Experimental Details of Electrophoresis of LACTATE DEHYDROGENASE in *Mugil cephalus* L.

Sl. No.	Electrophoretic Buffer Systems **	Tissues	Relative Electrophoretic Mobility (mm) and Intensity (x) of Different Bands						Total No. of Bands	Nature of Separation and Resolution
			1		2		3			
I	BOR	S	20-22	1x					1	P
		M	17-18	3x					1	G
		E	18-20	3x	23-24	3x			2	G
		H	17-18	1x					1	P
		L	19-21	1x					1	P
		I							0	
		K	18-19	1x					1	P
II	TCT	S	7-10	1x					1	P
		M	2-3	1x	9-10	1x	14-15	1x	3	P
		E	8-11	2x	18-19	2x			2	G
		H	9-12	1x					1	P
		L	9-12	2x					1	P
		I							0	
		K	8-12	2x					1	P
III	TGH	S	1-2	1x	14-18	3x	27-29	1x	3	G
		M	13-17	3x					1	B
		E	14-18	3x	23-25	1x	31-33	3x	3	B
		H	10-25	3x					1	P
		L	14-17	3x	35-37	1x	41-44	1x	3	G
		I	5-8	1x	31-33	1x	41-44	1x	3	P
		K	16-20	3x					1	G
IV	TVB	S	9-10	1x					1	P
		M	4-7	1x	8-9	1x			2	P
		E	8-9	1x	10-11	1x	16-18	1x	3	P
		H	9-11	3x					1	G
		L	10-11	2x					1	G
		I							0	
		K	10-11	3x					1	G

* Refer Page xvi for explanation of abbreviations

** Refer Table 2 for buffer systems

FIG. 42

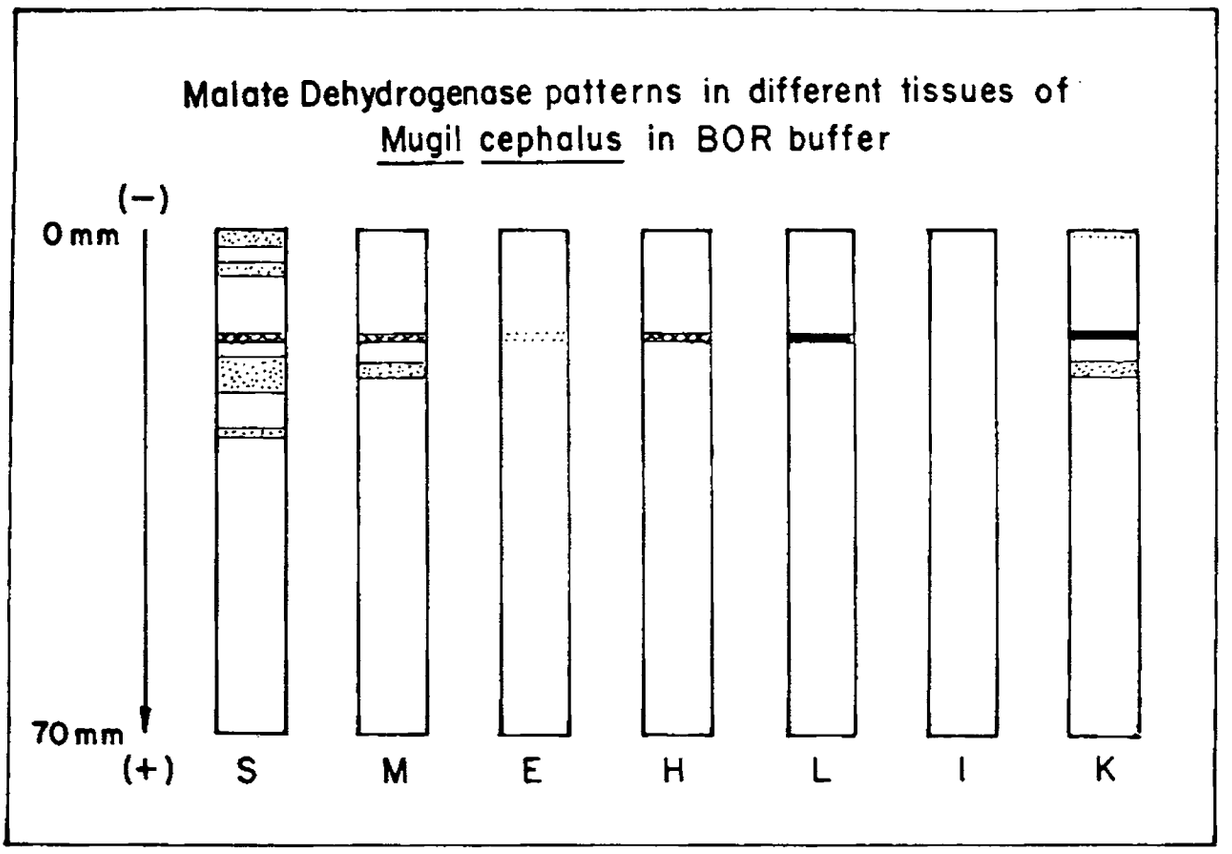

Malate Dehydrogenase patterns in different tissues of
Mugil cephalus in BOR buffer
(−)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

FIG. 43

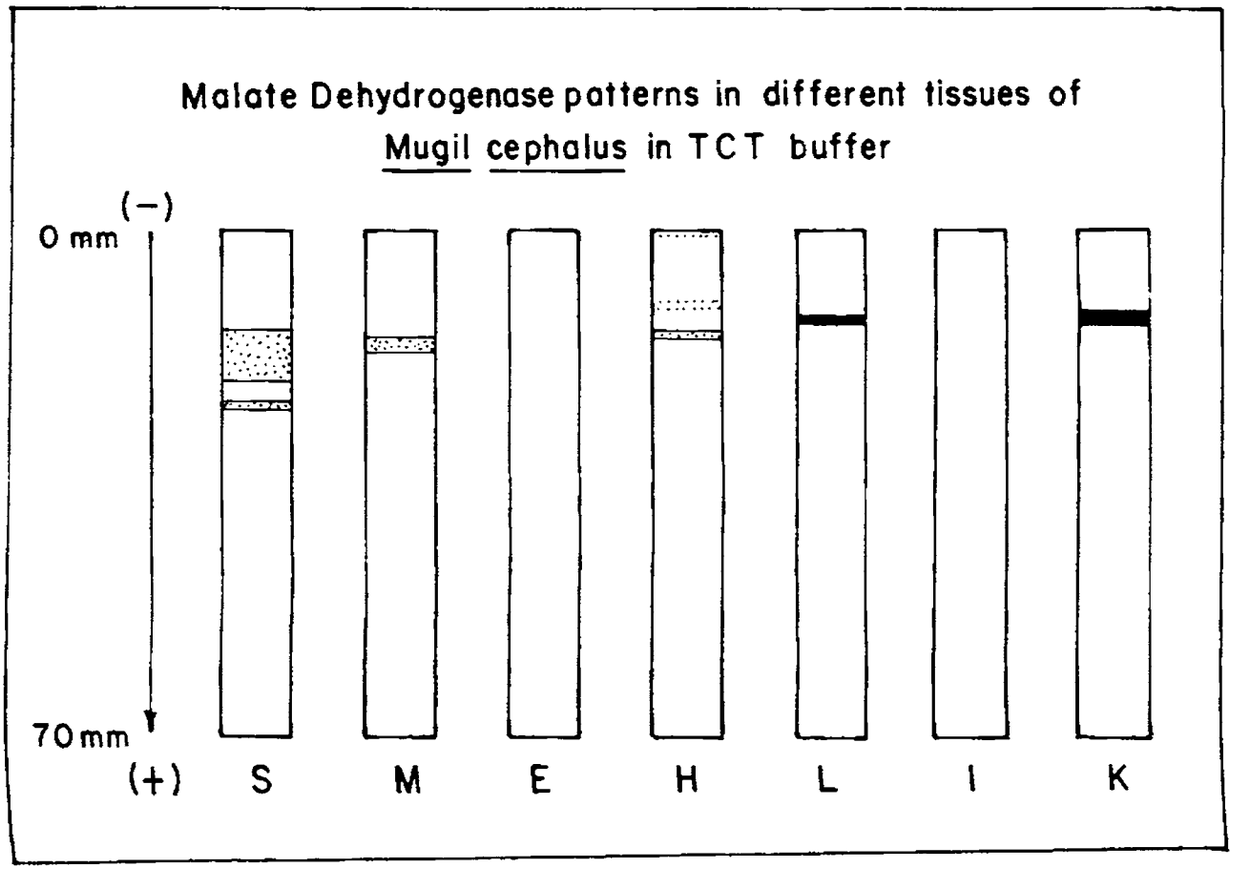

Malate Dehydrogenase patterns in different tissues of
Mugil cephalus in TCT buffer
(−)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

FIG. 44

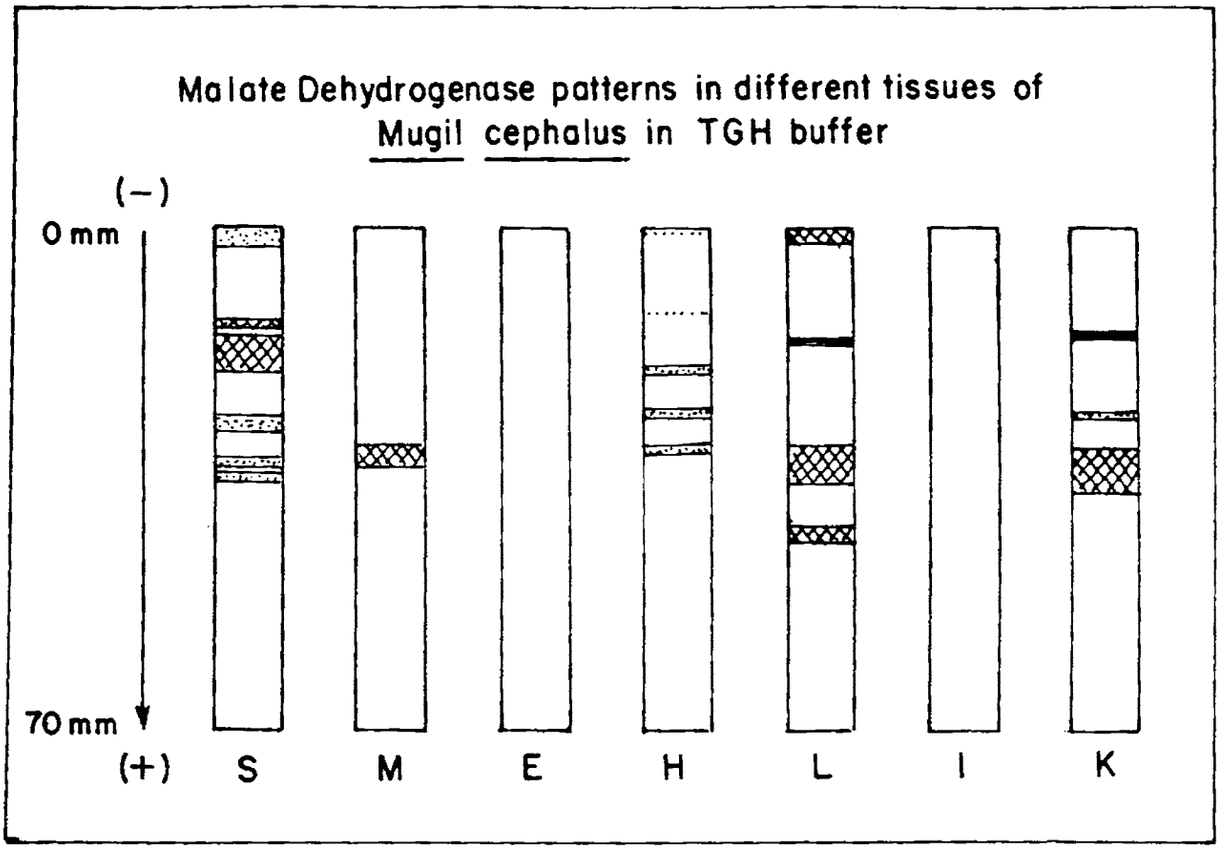

Malate Dehydrogenase patterns in different tissues of
Mugil cephalus in TGH buffer
(−)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

FIG. 45

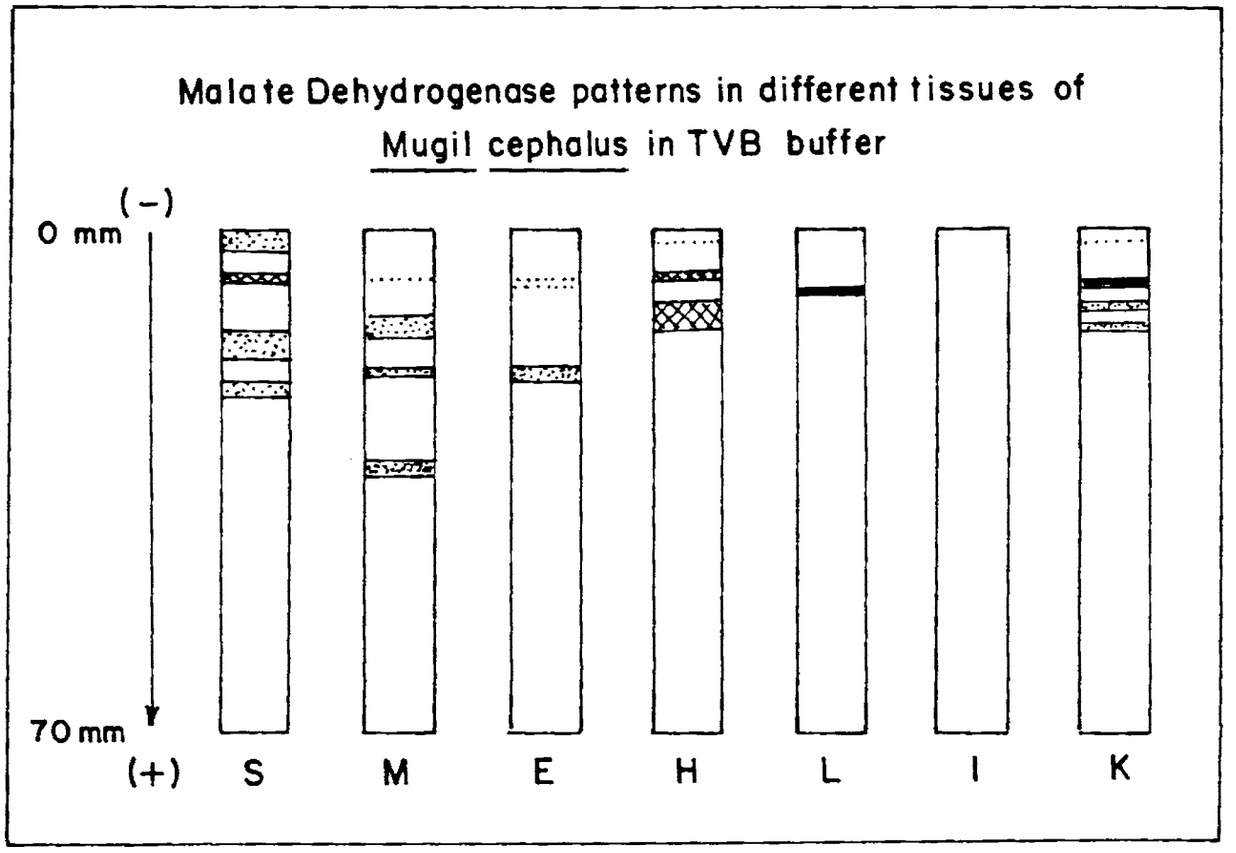

Malate Dehydrogenase patterns in different tissues of
Mugil cephalus in TVB buffer
(−)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

PLATE 12

Malate Dehydrogenase patterns in different tissues of M. cephalus in

A. Borate buffer

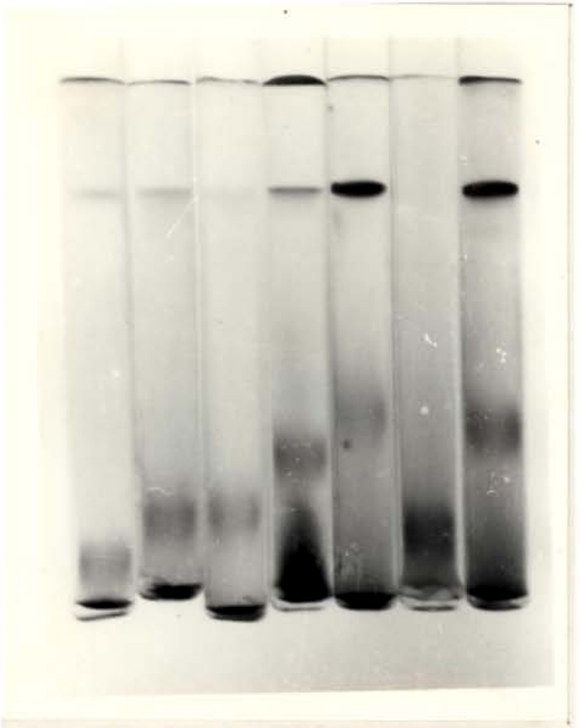
B. Tris Citrate buffer

C. Tris Glycine HCl buffer

D. Tris Versene Borate buffer

PLATE 12

(A) MDH-BOR

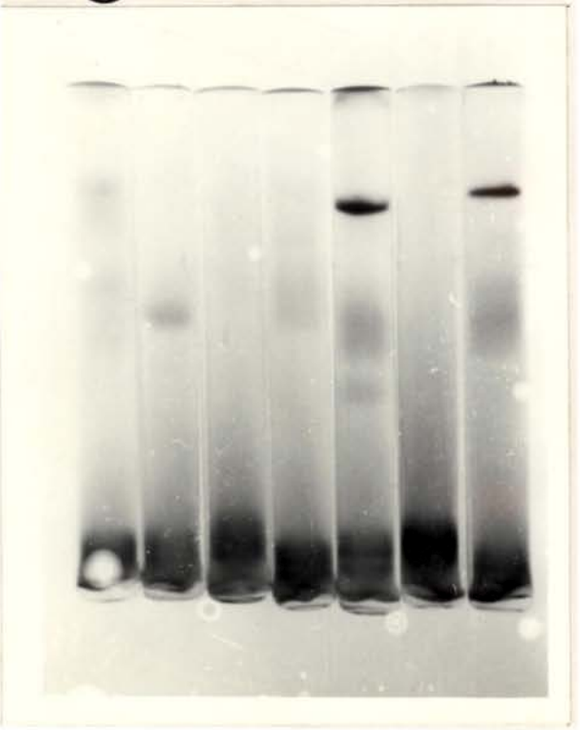


S M E H L I K

(B) MDH-TCT

S M E H L I K

(C) MDH-TGH



S M E H L I K

(D) MDH-TVB

S M E H L I K

TABLE 14*

Experimental Details of Electrophoresis of MALATE DEHYDROGENASE in

Mugil cephalus L.

Sl. No.	Electrophoretic Buffer Systems **	Tissues	Relative Electrophoretic Mobility (mm) and Intensity (x) of Different Bands												Total No. of Bands	Nature of Separation and Resolution
			1		2		3		4		5		6			
I	BOR	S	0-2	1x	4-6	1x	14-15	2x	17-22	1x	27-28	1x			5	P
		M	14-15	2x	18-20	1x									2	P
		E	14	1x	15	1x									2	P
		H	14-15	2x											1	G
		L	14-15	3x											1	G
		I													0	
		K	1	1x	14-15	3x	18-20	1x							3	G
II	TCT	S	14-21	1x	24-25	1x									2	P
		M	15-17	1x											1	P
		E													0	
		H	1	1x	10	1x	11	1x	14-15	1x					4	P
		L	12-13	3x											1	B
		I													0	
		K	11-13	3x											1	G
III	TGH	S	0-3	1x	13-14	2x	15-20	2x	26-28	1x	32-33	1x	34-35	1x	6	P
		M	30-33	2x											1	P
		E													0	
		H	1	1x	12	1x	19-20	1x	25-26	1x	30-31	1x			5	P
		L	0-2	2x	15-16	3x	30-35	2x	41-43	2x					4	G
		I													0	
		K	14-15	3x	25-26	1x	30-36	2x							3	G
IV	TVB	S	0-3	1x	6-7	2x	14-18	1x	21-23	1x					4	P
		M	7	1x	12-15	1x	19-20	1x	32-34	1x					4	P
		E	7	1x	8	1x	19-21	1x							3	P
		H	2	1x	6-7	2x	10-14	2x							3	P
		L	8-9	3x											1	G
		I													0	
		K	2	1x	7-8	3x	10-11	1x	13-14	1x					4	G

* Refer Page xvi for explanation of abbreviations

** Refer Table 2 for buffer systems

FIG. 46

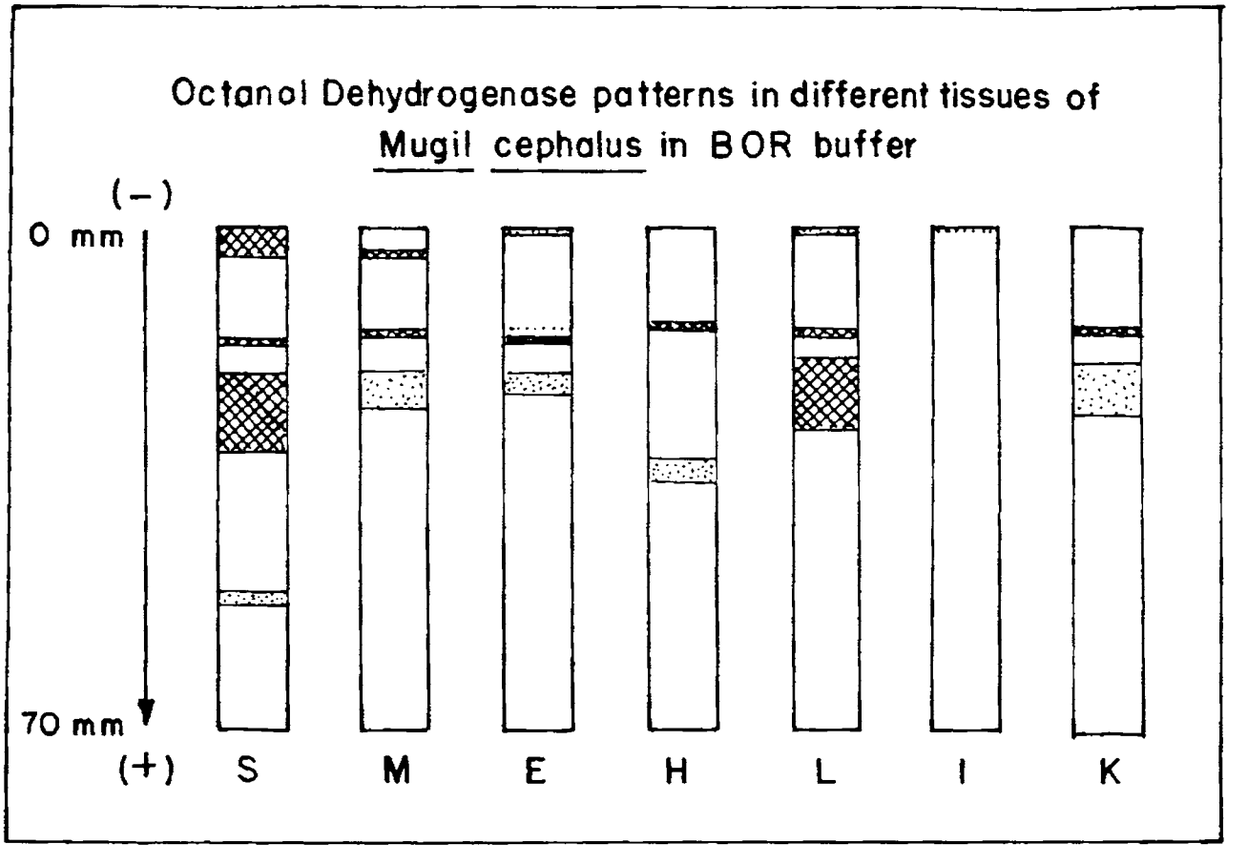

Octanol Dehydrogenase patterns in different tissues of
Mugil cephalus in BOR buffer
(−)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

FIG. 47

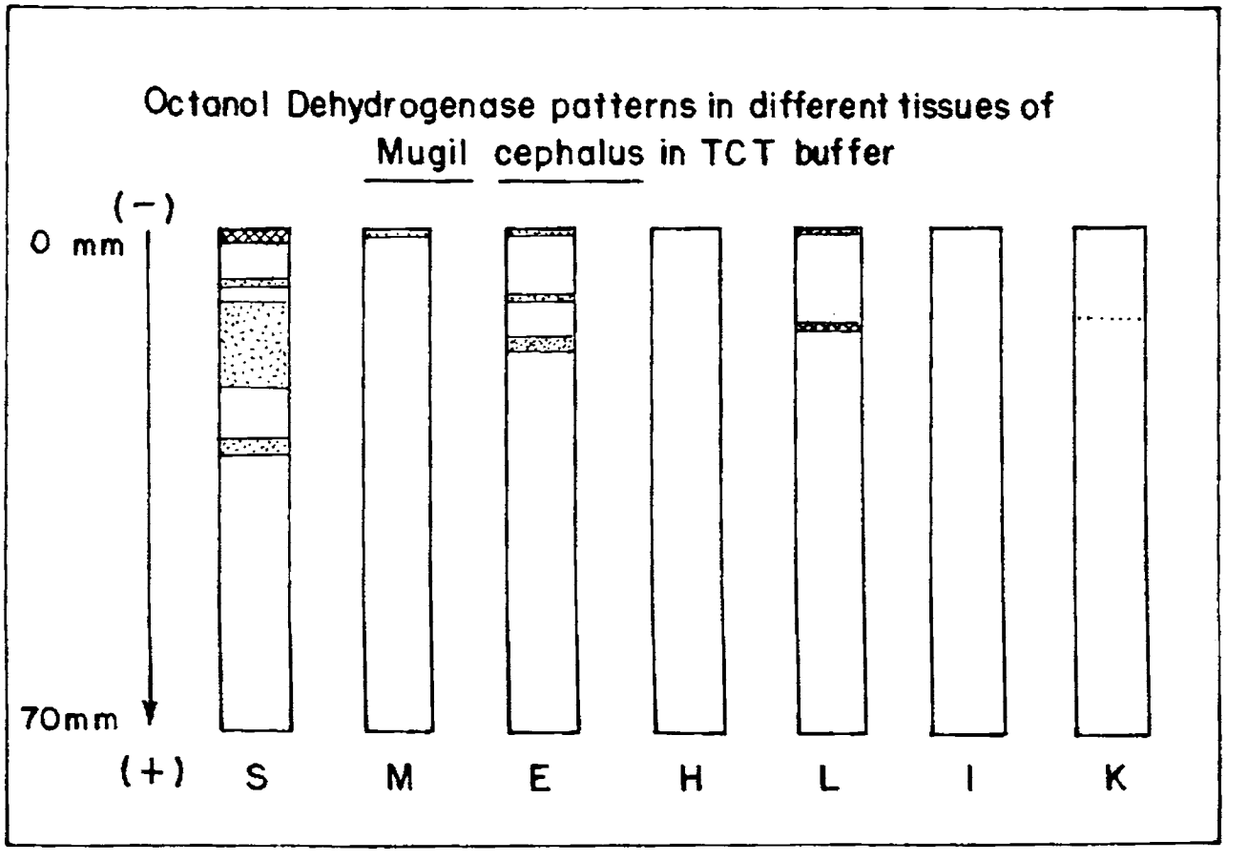

Octanol Dehydrogenase patterns in different tissues of
Mugil cephalus in TCT buffer
(−)
0 mm
70mm
(+)
S
M
E
H
L
I
K

FIG. 48

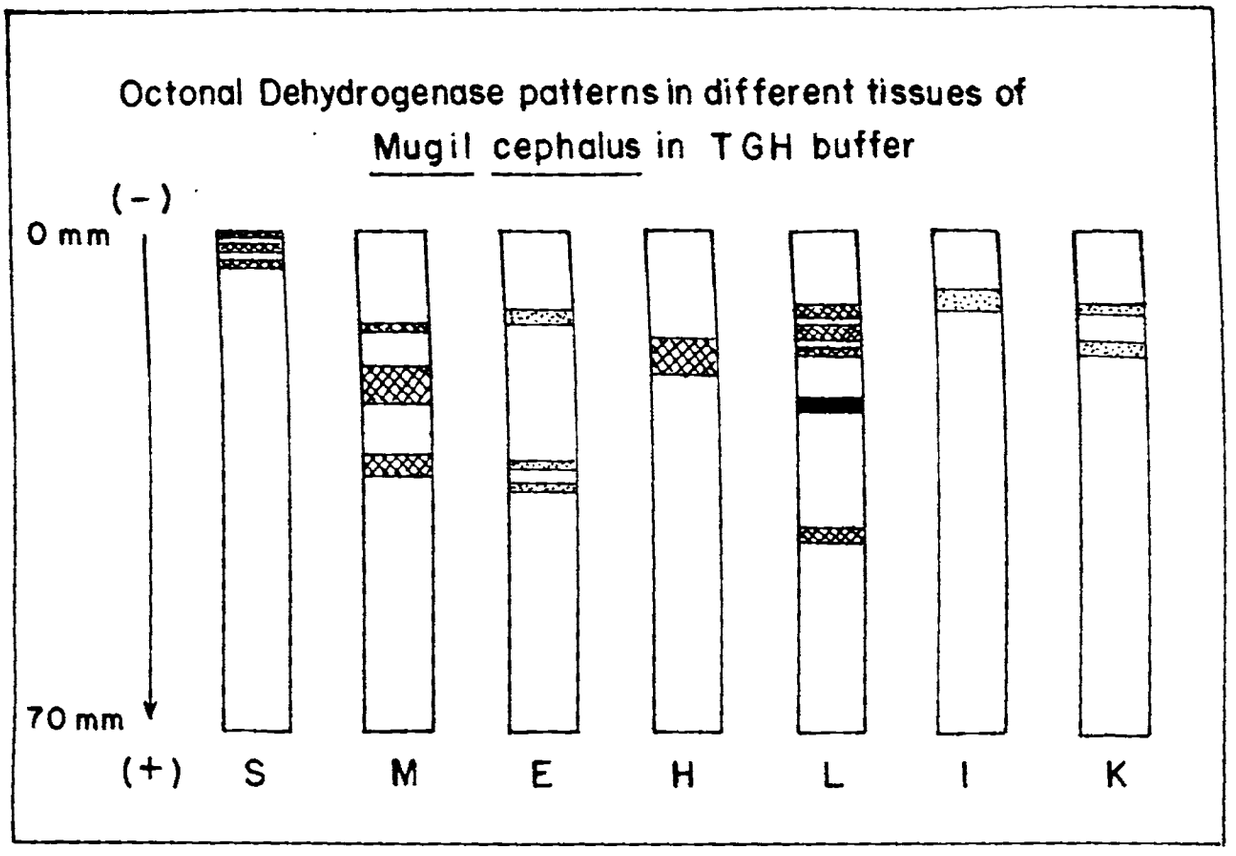

Octonal Dehydrogenase patterns in different tissues of
Mugil cephalus in TGH buffer
(−)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

FIG. 49

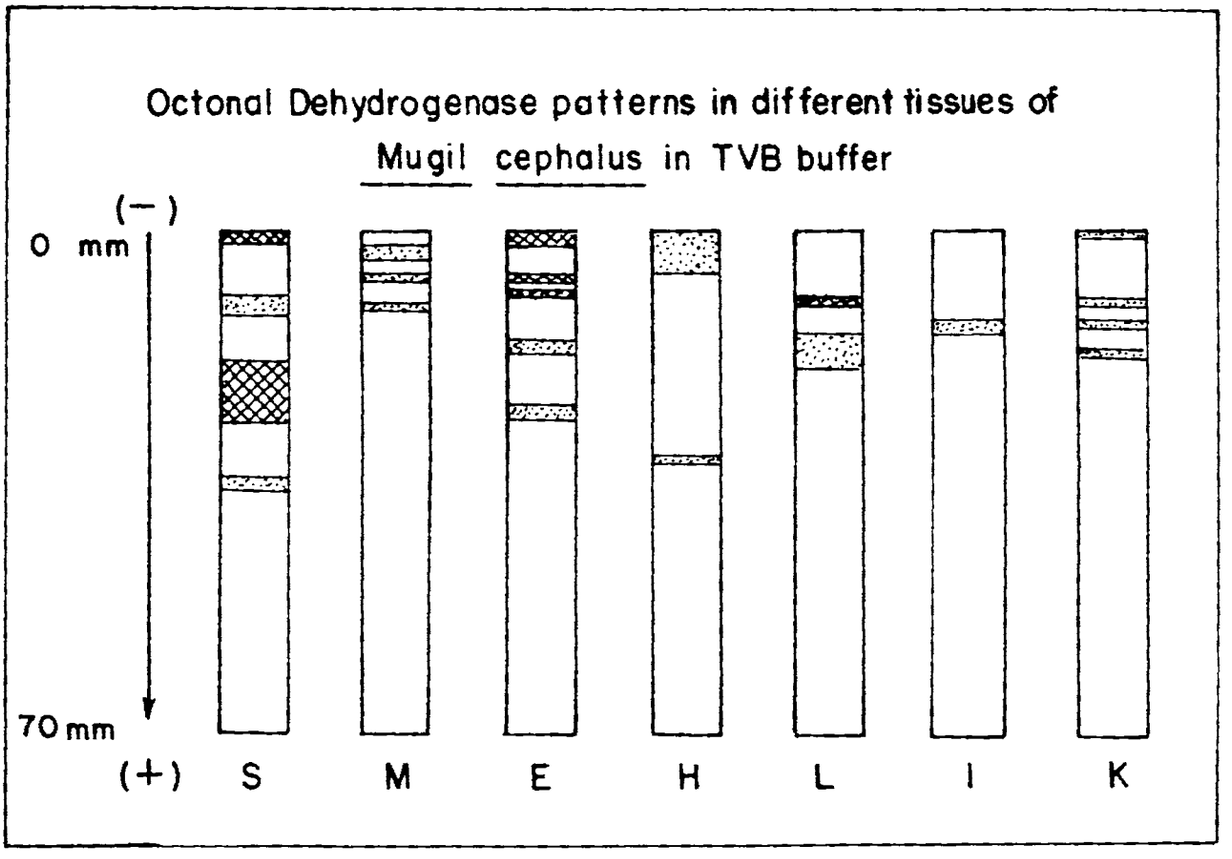

Octonal Dehydrogenase patterns in different tissues of
Mugil cephalus in TVB buffer
(−)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

table 15*

Experimental Details of Electrophoresis of OCTANOL DEHYDROGENASE in

Mugil cephalus L.

Sl. No.	Electrophoretic Buffer Systems **	Tissues	Relative Electrophoretic Mobility (mm) and Intensity (x) of Different Bands					Total No.of Bands	Nature of Separation and Resolution
			1	2	3	4	5		
I	BOR	S	0-4 2x	15-16 2x	20-31 2x	50-52 1x		4	G
		M	3-4 2x	14-15 2x	20-25 1x			3	G
		E	0-1 1x	14 1x	15-16 3x	20-23 1x		4	G
		H	13-14 2x	32-35 1x				2	G
		L	0-1 2x	14-15 2x	18-28 2x			3	G
		I	1 1x					1	P
		K	14-15 2x	19-26 1x				2	G
II	TCT	S	0-2 2x	7-8 1x	10-22 1x	29-31 1x		4	P
		M	0-1 1x					1	P
		E	0-1 1x	9-10 1x	15-17 1x			3	P
		H						0	
		L	0-1 2x	13-14 2x				2	G
		I						0	
		K	13 1x					1	P
III	TGH	S	0-1 2x	2-3 2x	4-5 2x			3	G
		M	13-14 2x	19-24 2x	31-34 2x			3	G
		E	11-13 1x	32-33 1x	35-36 1x			3	P
		H	15-20 2x					1	G
		L	10-12 2x	13-15 2x	16-17 2x	23-25 3x	41-44 2x	5	B
		I	8-11 1x					1	P
		K	10-11 1x	15-17 1x				2	P
IV	TVB	S	0-2 2x	9-12 1x	18-27 2x	44-46 1x		4	P
		M	2-4 1x	6-7 1x	10-11 1x			3	P
		E	0-2 2x	6-7 2x	8-9 2x	15-17 1x	24-26 1x	5	G
		H	0-6 1x	31-32 1x				2	P
		L	9-10 2x	14-19 1x				2	G
		I	12-14 1x					1	P
		K	0-1 1x	9-10 1x	12-13 1x	16-17 1x		4	G

* Refer Page xvi for explanation of abbreviations

** Refer Table 2 for buffer systems

FIG. 50

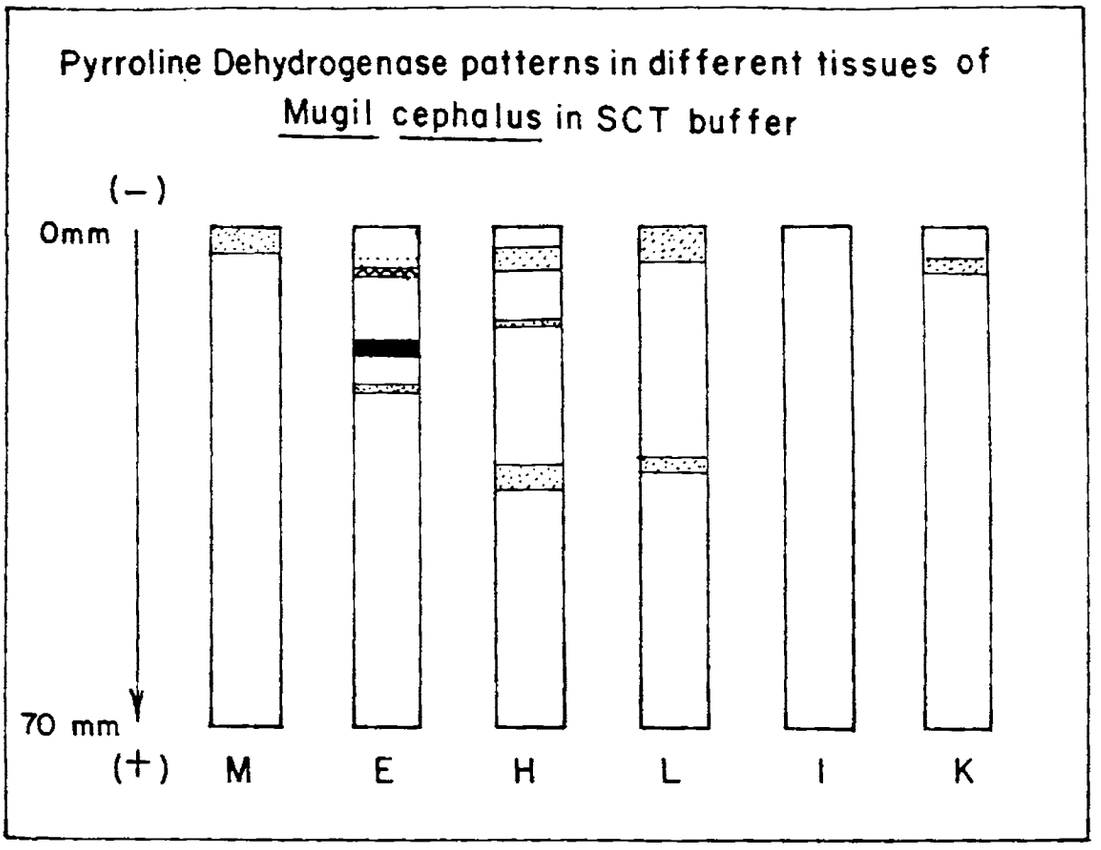

Pyrroline Dehydrogenase patterns in different tissues of
Mugil cephalus in SCT buffer
(−)
0mm
70 mm
(+)
M
E
H
L
I
K

FIG. 51

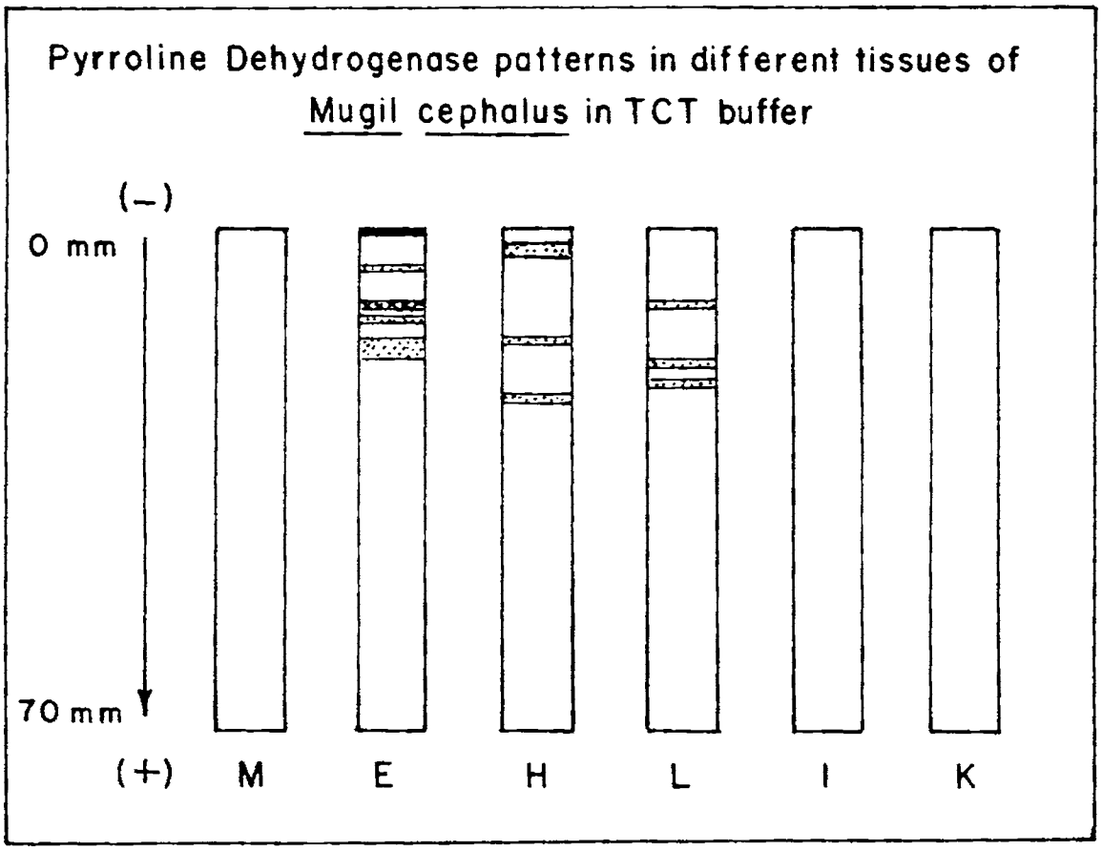

Pyrroline Dehydrogenase patterns in different tissues of
Mugil cephalus in TCT buffer
(−)
0 mm
70 mm
(+)
M
E
H
L
I
K

FIG. 52

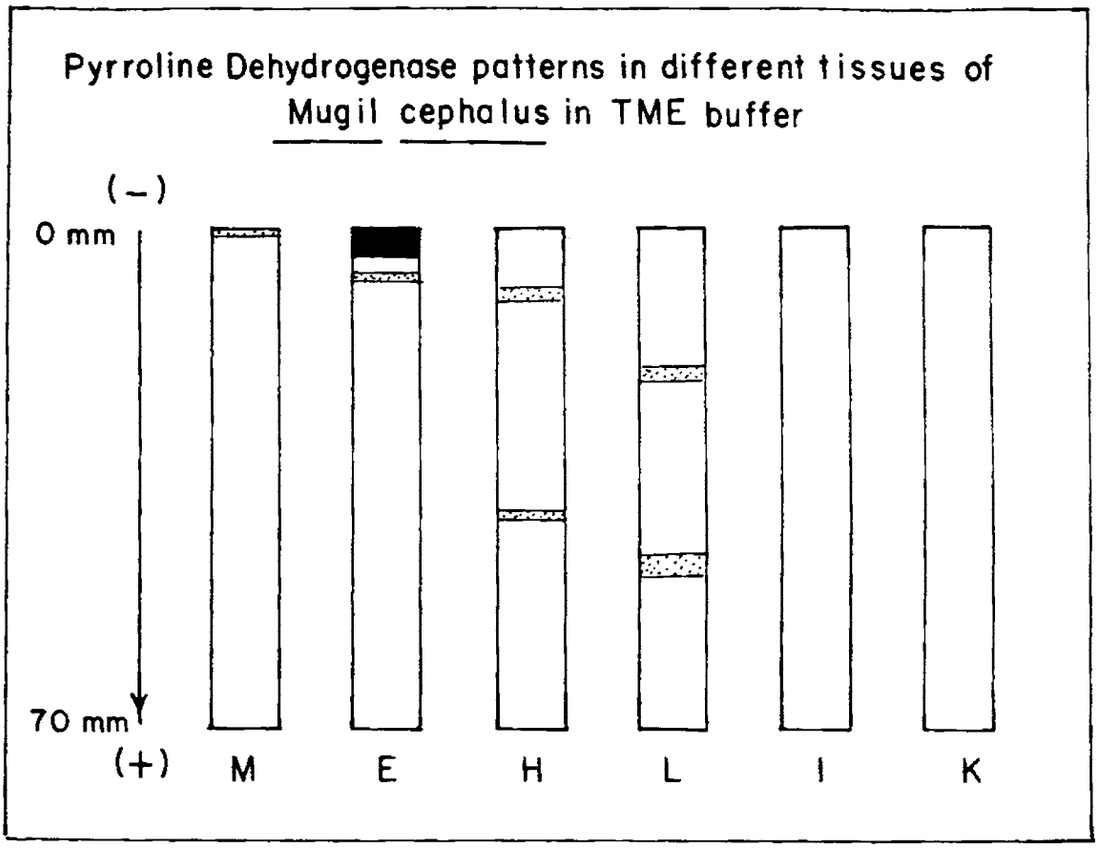

Pyrroline Dehydrogenase patterns in different tissues of
Mugil cephalus in TME buffer
(−)
0 mm
70 mm
(+)
M
E
H
L
I
K

FIG. 53

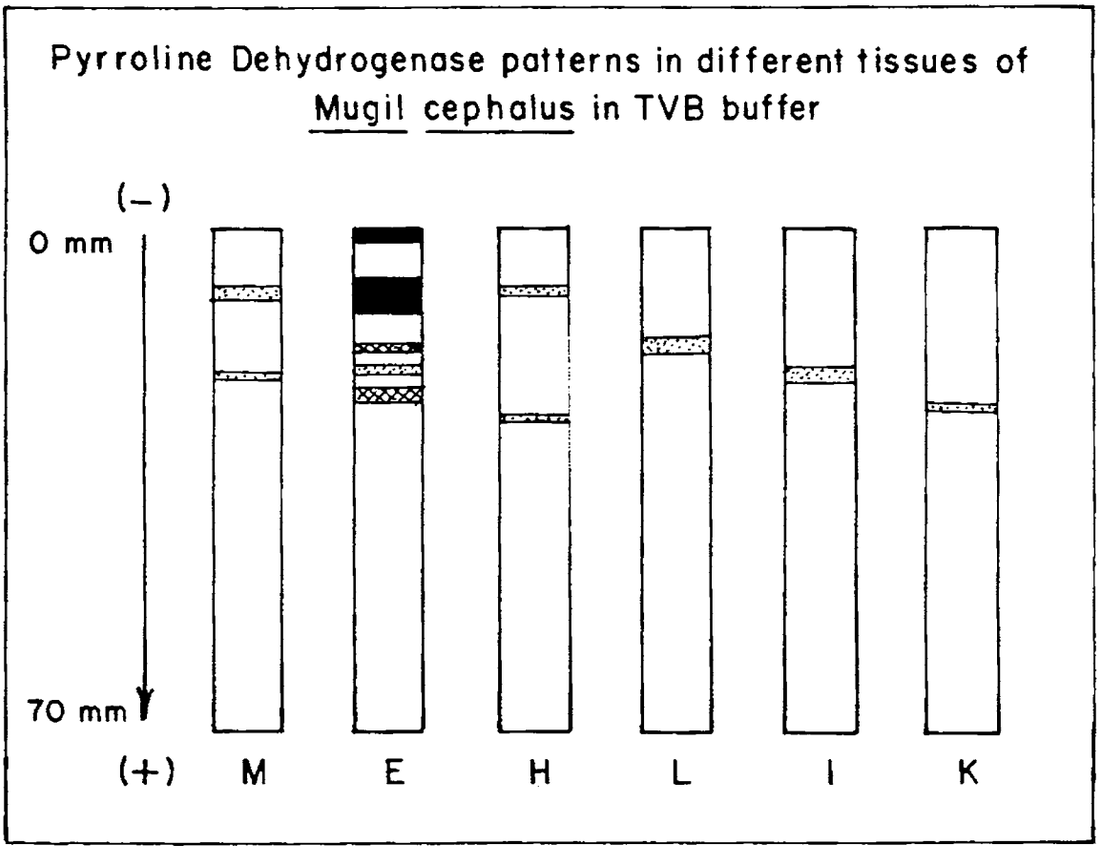

Pyrroline Dehydrogenase patterns in different tissues of
Mugil cephalus in TVB buffer
(−)
0 mm
70 mm
(+)
M
E
H
L
I
K

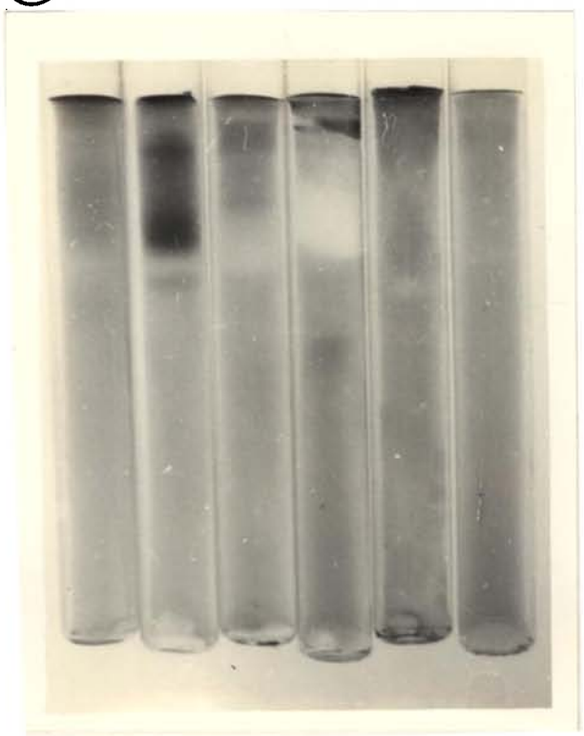
PLATE 13

Pyrroline Dehydrogenase patterns in different tissues of M. cephalus in

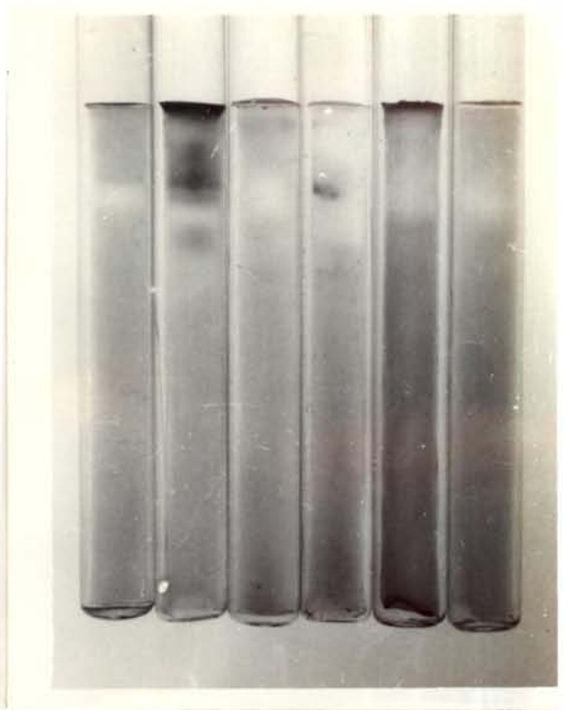
A. Sodium Citrate buffer
B. Tris Citrate buffer
C. Tris Maleic EDTA buffer
D. Tris Versene Borate buffer

PLATE 13

(A) PDH-SCT

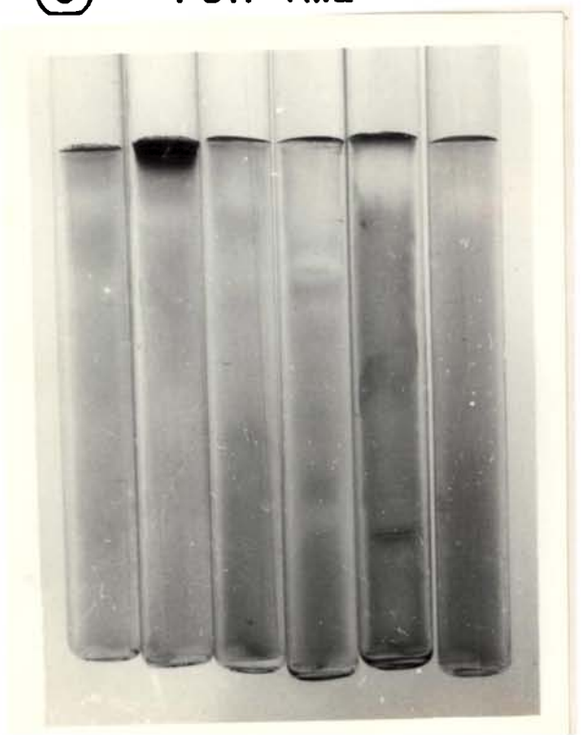


(B) PDH-TCT



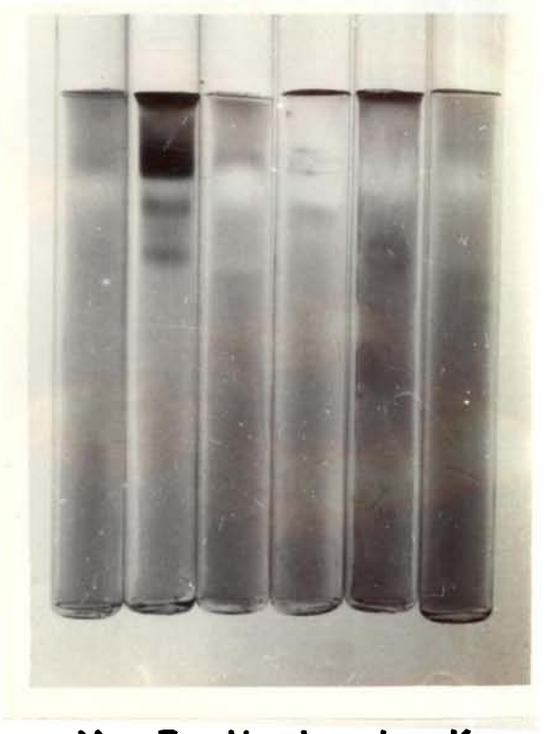
M E H L I K

(C) PDH-TME



M E H L I K

(D) PDH-TVB



M E H L I K

TABLE 16*

Experimental Details of Electrophoresis of PYRROLINE DEHYDROGENASE in *Mugil cephalus* L.

Sl. No.	Electrophoretic Buffer Systems **	Tissues	Relative Electrophoretic Mobility (mm) and Intensity (x) of Different Bands					Total No. of Bands	Nature of Separation and Resolution
			1	2	3	4	5		
I	SCT	M	0-4 1x					1	P
		E	5 1x	6-7 2x	15-18 3x	22-23 1x		4	G
		H	3-6 1x	13-14 1x	33-36 1x			3	G
		L	0-5 1x	32-34 1x				2	P
		I						0	
		K	4-6 1x					1	P
II	TCT	M						0	
		E	0-1 3x	5-6 1x	10-11 2x	12-13 1x	15-18 1x	5	G
		H	2-4 1x	15-16 1x	23-24 1x			3	P
		L	10-11 1x	18-19 1x	21-22 1x			3	P
		I						0	
		K						0	
III	TME	M	0-1 1x					1	P
		E	0-4 3x	6-7 1x				2	G
		H	8-10 1x	39-40 1x				2	P
		L	19-21 1x	45-48 1x				2	P
		I						0	
		K						0	
IV	TVB	M	8-10 1x	20-21 1x				2	P
		E	0-2 3x	7-12 3x	16-17 2x	19-20 1x	22-24 1x	5	B
		H	8-9 1x	26-27 1x				2	G
		L	15-17 1x					1	G
		I	19-21 1x					1	P
		K	24-25 1x					1	P

* Refer page xvi for explanation of abbreviations

** Refer Table 2 for buffer systems

FIG. 54

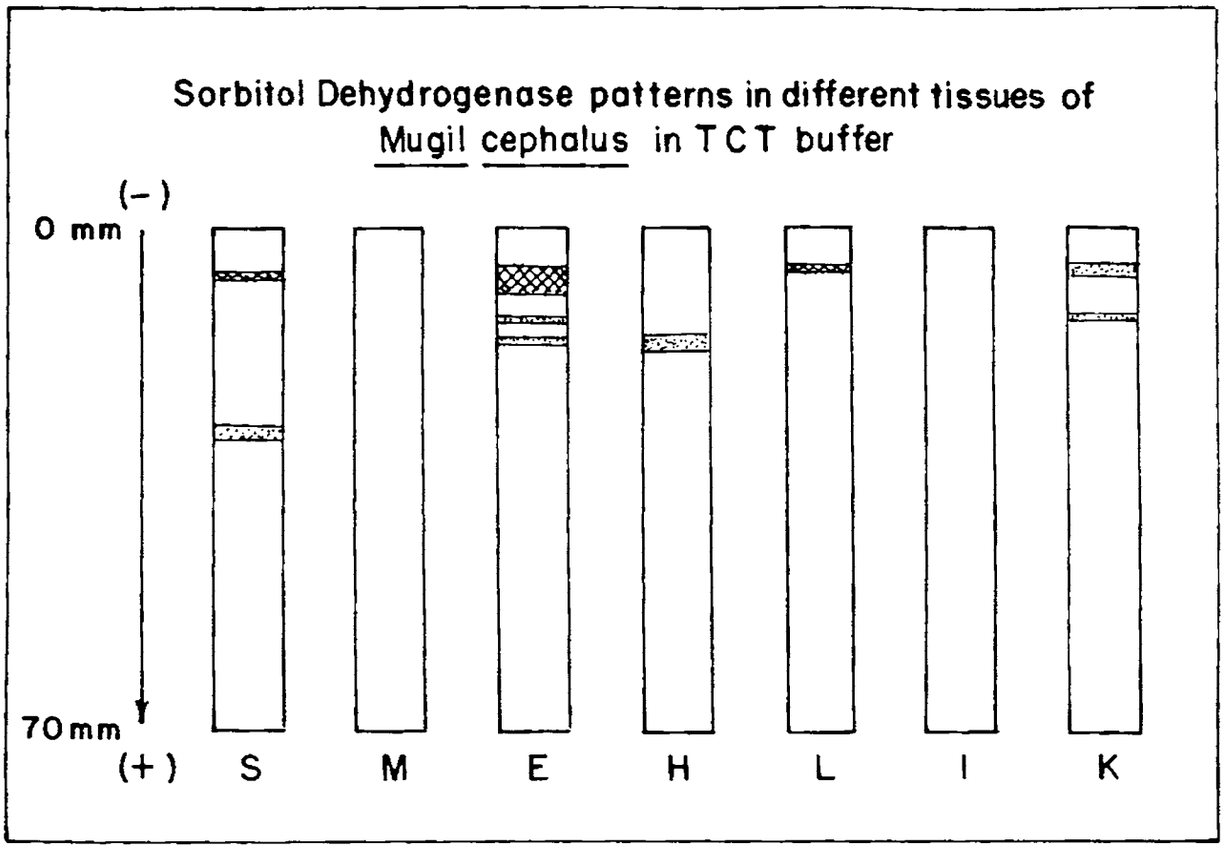

Sorbitol Dehydrogenase patterns in different tissues of
Mugil cephalus in TCT buffer
(-)
0 mm
70mm
(+)
S
M
E
H
L
I
K

FIG. 55

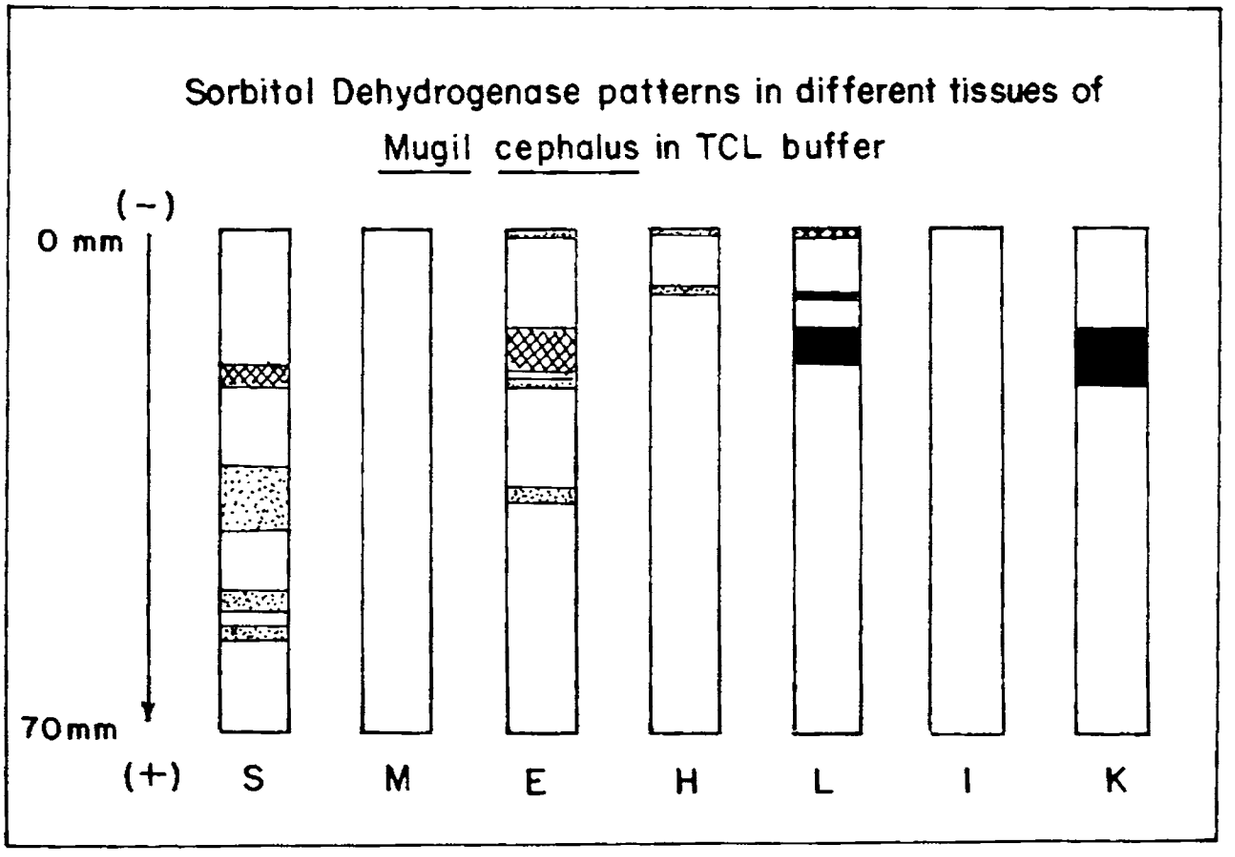

Sorbitol Dehydrogenase patterns in different tissues of
Mugil cephalus in TCL buffer
(-)
0 mm
70mm
(+)
S
M
E
H
L
I
K

FIG. 56

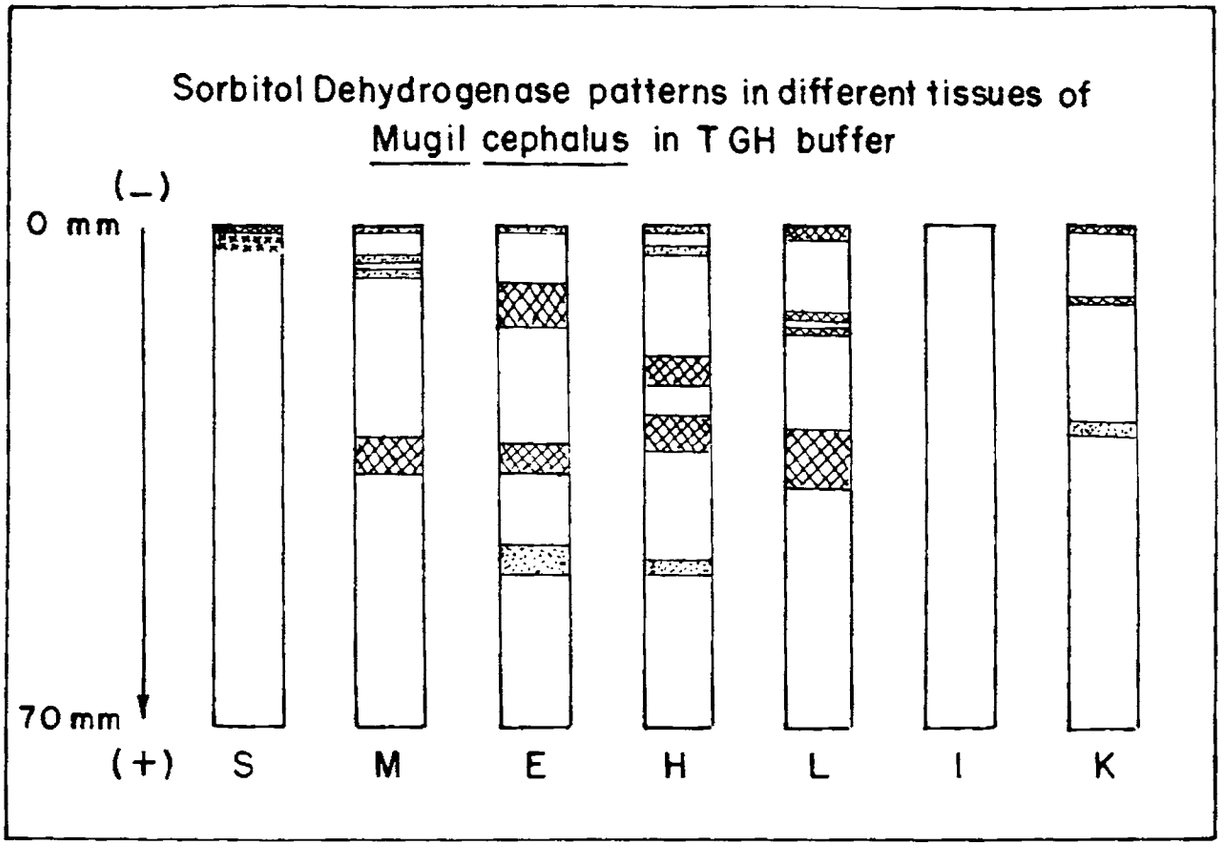

Sorbitol Dehydrogenase patterns in different tissues of
Mugil cephalus in T GH buffer
(–)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

FIG. 57

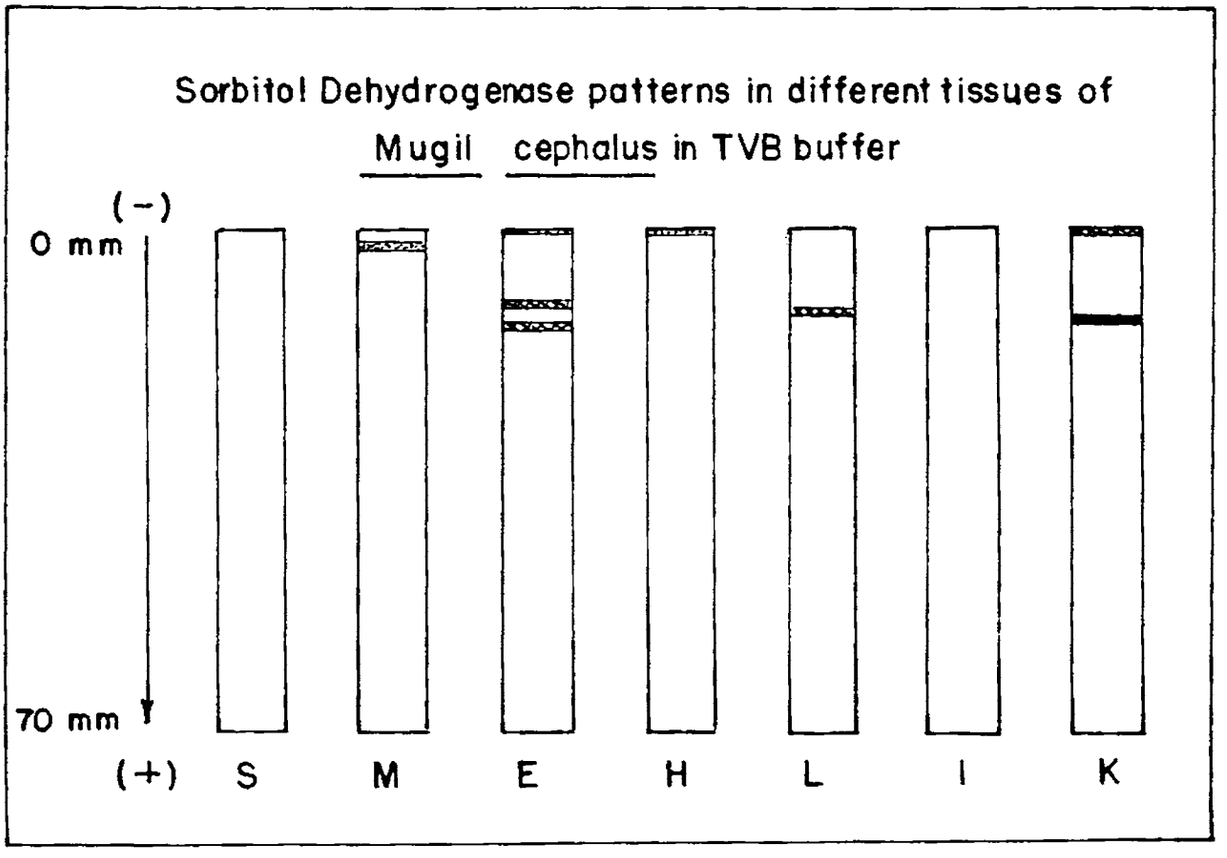

Sorbitol Dehydrogenase patterns in different tissues of
Mugil cephalus in TVB buffer
(–)
0 mm
70 mm
(+)
S
M
E
H
L
I
K

TABLE 17*

Experimental Details of Electrophoresis of SORBITOL DEHYDROGENASE in *Mugil cephalus* L.

Sl. No.	Electrophoretic Buffer Systems **	Tissues	Relative Electrophoretic Mobility (mm) and Intensity (x) of Different Bands										Total No. of Bands	Nature of Separation and Resolution
			1		2		3		4		5			
I	TCT	S	6-7	2x	27-29	1x							2	G
		M											0	
		E	5-9	2x	12-13	1x	15-16	1x					3	P
		H	15-17	1x									1	P
		L	5-6	2x									1	G
		I											0	
		K	5-7	1x	12-13	1x							2	G
II	TCL	S	19-22	2x	34-42	1x	50-53	1x	55-57	1x			4	G
		M											0	
		E	0-1	1x	14-20	2x	21-22	1x	36-38	1x			4	G
		H	0-1	1x	8-9	1x							2	P
		L	0-1	2x	9-10	3x	14-19	3x					3	B
		I											0	
		K	14-22	3x									1	G
III	TGH	S	0-1	2x	2	2x	3	2x					3	G
		M	0-1	1x	4-5	1x	6-7	1x	29-34	2x			4	G
		E	0-1	1x	8-14	2x	30-34	2x	44-48	1x			4	G
		H	0-1	1x	3-4	1x	18-22	2x	26-31	2x	46-48	1x	5	G
		L	0-2	2x	12-13	2x	14-15	2x	28-36	2x			4	G
		I											0	
		K	0-1	2x	10-11	2x	27-29	1x					3	G
IV	TVB	S											0	
		M	2-3	1x									1	G
		E	0-1	1x	10-11	2x	13-14	2x					3	G
		H	0-1	1x									1	P
		L	11-12	2x									1	G
		I											0	
		K	0-1	2x	12-13	3x							2	G

* Refer page xvi for explanation of abbreviations

** Refer Table 2 for buffer systems

ratios taken for ACP, GPD and MDH were 2.5 ml and 2 ml respectively. For ADH, ADO, ALD, AKP, GDH, GOT, LDH (M), ODH, PDH, SDH and TZO these gel proportions were 3.5 ml and 2 ml respectively. However, a combinations of 4.5 ml and 3 ml was necessary to produce good patterns of LDH(E) enzyme and 5 ml and 3 ml for esterase enzyme. For general protein 7.5% gel made as described in materials and methods was used for obtaining good separation of different protein components. Similarly, for majority of enzymes, a sample concentration of 100 mg tissue/ml was necessary to give good results whereas for EST, GDH, LDH(E), PDH and PRO(E) only 40 mg/ml was necessary. For PRO(M) 100 mg/ml was required to show better patterns. Liver tissue produced comparatively better patterns in majority of enzymes tested. However, heart was found to be the best source of esterase pattern whereas eye lens produced better patterns for GDH, LDH(E) and PDH. The pH mentioned in the Table 2 was the required pH for the Electrode and Gel buffers for each enzyme system.

4.2 SPECIES SPECIFIC PROTEIN PATTERNS

A comparison of zymogram patterns of fifteen enzyme systems and two general protein systems among M. cephalus, L. parsia and V. cunnesius from Cochin showed distinct species specific biochemical genetic patterns for each species (FIG. 58A - 62C; PLT. 14A - 18B). Though all these individual zymograms were species specific in one or more respects of electrophoretic characteristics, these may be compared in three different groups. In the first group, differences are shown by distinct patterns of a particular protein system in each species. For example, patterns of ACP, ADH, GPD and SDH are distinctly different in each species in terms of either number of bands or their gel position or by both (FIG. 58A, B, 59D, 62B; PLT. 14A, B, 15D, 18B; TBL. 18). The second group is composed of such zymogram patterns which show distinct species specific patterns between any two species (FIG. 58C, D, 59A-C, 60A-D, 61A, B, 62A, C; PLT. 14C, D, 15A-C, 16A-D, 17A, B, 18A; TBL. 18). The zymogram patterns that belong to this group are ADO between species 1 and 2, 3; ALD between species 1 and 2, 3; AKP between species 2 and 1, 3; EST between species 3 and 1, 2; eye lens GDH between

FIGURE 58A AND PLATE 14A

Liver Acid Phospharase patterns in

1. M. cephalus
2. L. parsia
3. V. cunnesius

FIGURE 58B AND PLATE 14B

Liver Alcohol Dehydrogenase patterns in

1. M. cephalus
2. L. parsia
3. V. cunnesius

FIGURE 58C AND PLATE 14C

Liver Aldehyde Oxidase patterns in

1. M. cephalus
2. L. parsia
3. V. cunnesius

FIGURE 58D AND PLATE 14D

Liver Aldolase patterns in

1. M. cephalus
2. L. parsia
3. V. cunnesius

FIG. 58

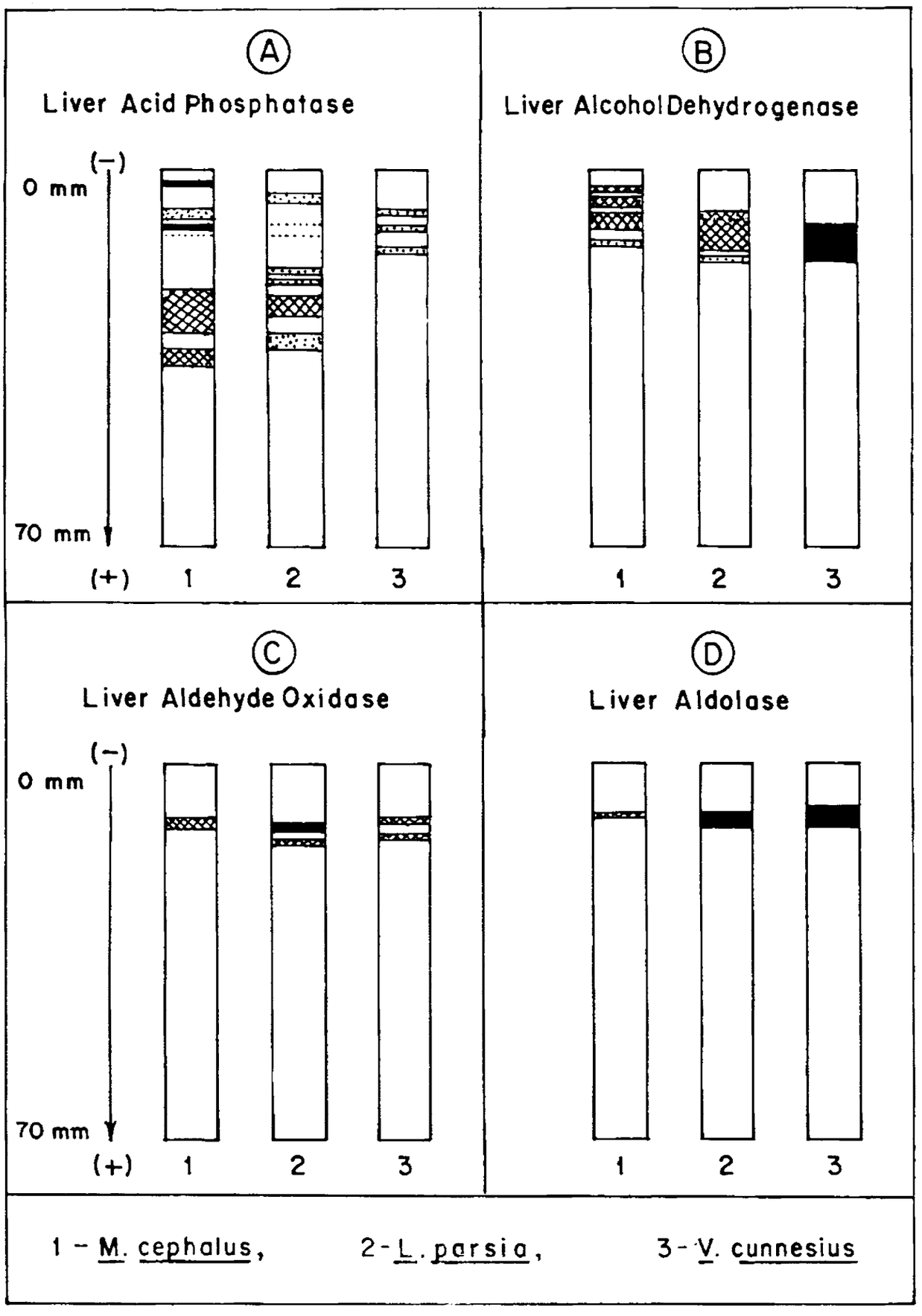
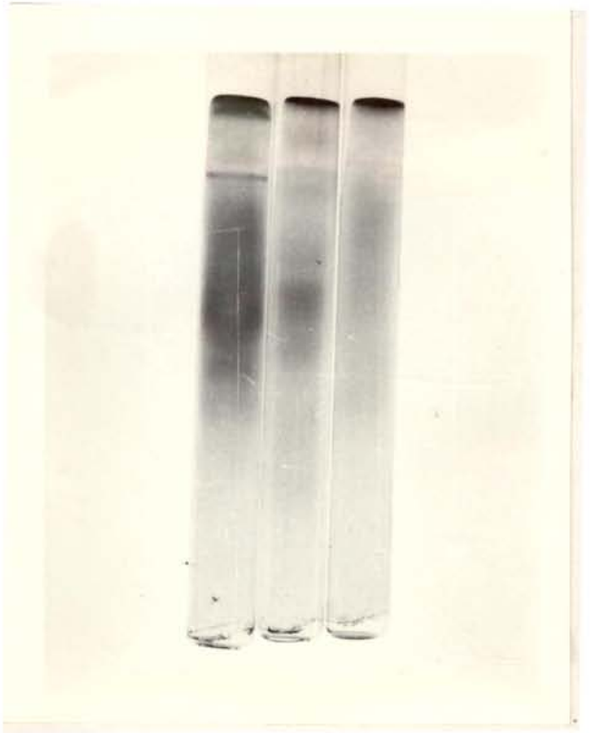

A
Liver Acid Phosphatase
(−)
0 mm
70 mm
(+)
1
2
3
B
Liver Alcohol Dehydrogenase
1
2
3
C
Liver Aldehyde Oxidase
(−)
0 mm
70 mm
(+)
1
2
3
D
Liver Aldolase
1
2
3
1 - M. cephalus, 2 - L. parsia, 3 - V. cunnesius

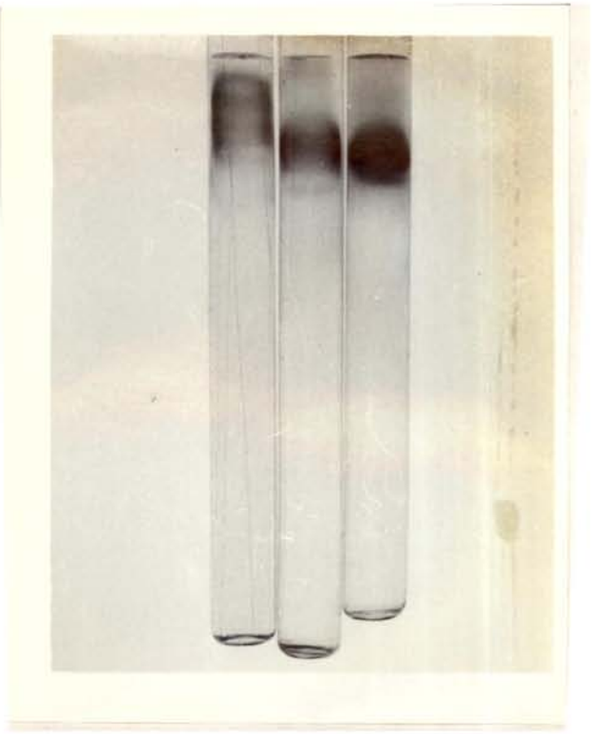
PLATE 14

(A) LIVER-ACP



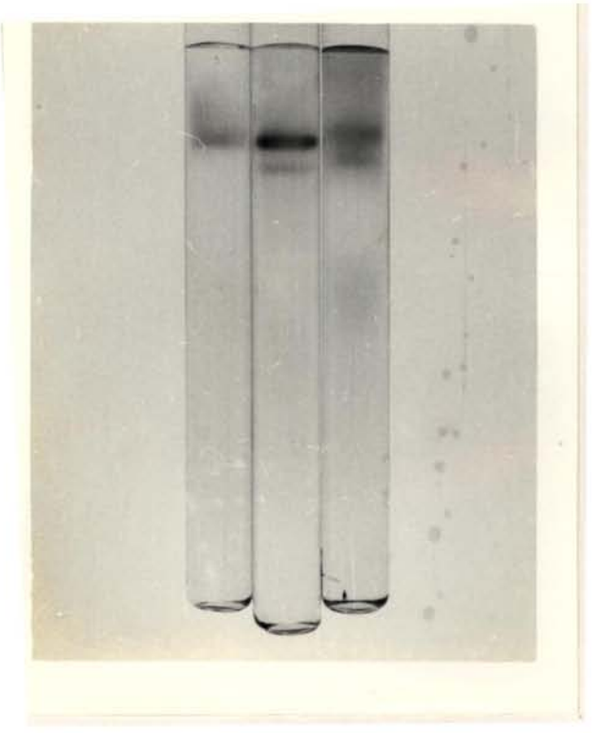
1 2 3

(B) LIVER-ADH



1 2 3

(C) LIVER-ADO



1 2 3

(D) LIVER-ALD

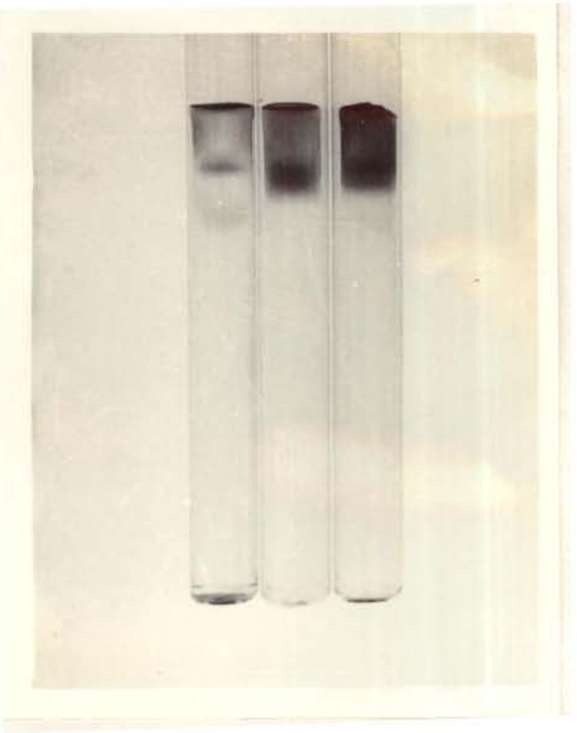


FIGURE 59A AND PLATE 15A

Liver Alkaline Phosphatase patterns in

1. M. cephalus
2. L. parsia
3. V. cunnesius

FIGURE 59B AND PLATE 15B

Heart Esterase patterns in

1. M. cephalus
2. L. parsia
3. V. cunnesius

FIGURE 59C AND PLATE 15C

Eye lens Glutamate Dehydrogenase patterns in

1. M. cephalus
2. L. parsia
3. V. cunnesius

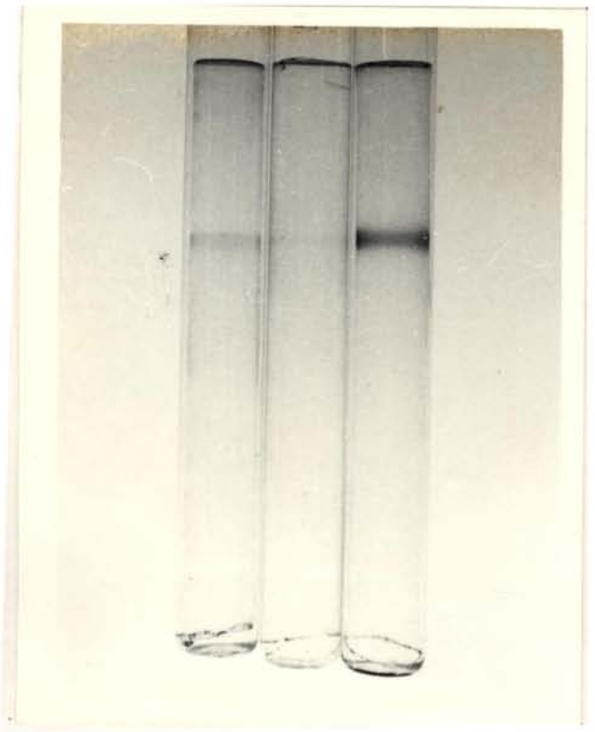
FIGURE 59D AND PLATE 15D

Liver Alpha-Glycerophosphate Dehydrogenase patterns in

1. M. cephalus
2. L. parsia
3. V. cunnesius

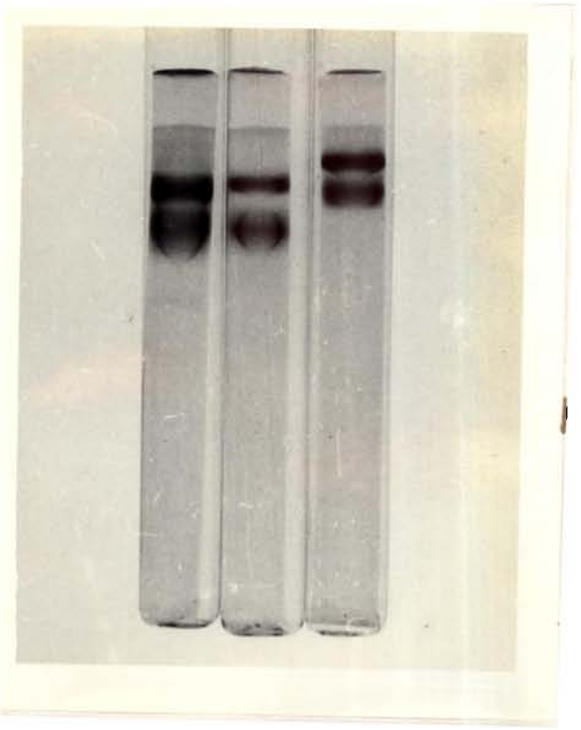
PLATE 15

(A) LIVER-AKP



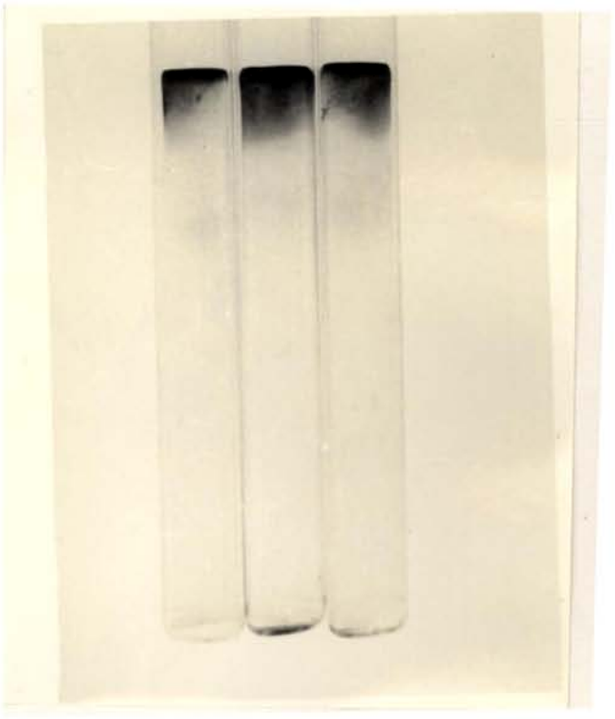
1 2 3

(B) HEART-EST



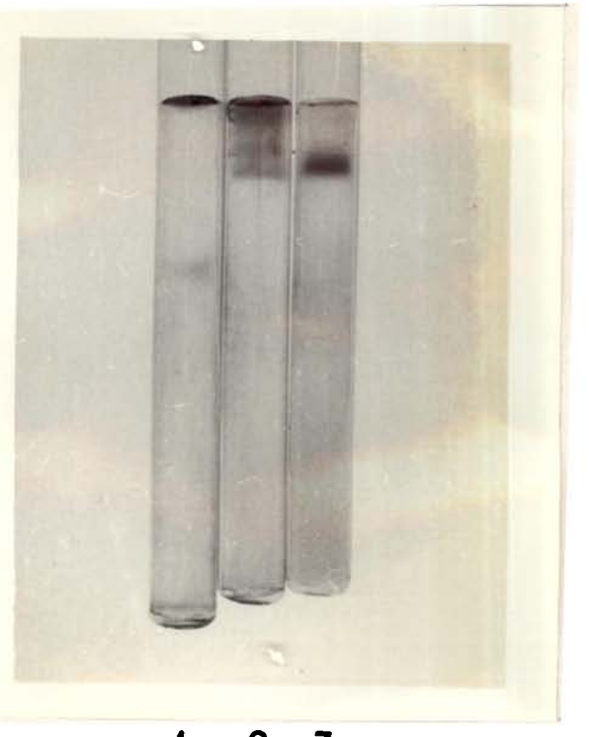
1 2 3

(C) EYE LENS-GDH



1 2 3

(D) LIVER-GPD



1 2 3

FIGURE 60A AND PLATE 16A

Eye lens Lactate Dehydrogenase patterns in

1. M. cephalus
2. L. parsia
3. V. cunnesius

FIGURE 60B AND PLATE 16B

Muscle Lactate Dehydrogenase patterns in

1. M. cephalus
2. L. parsia
3. V. cunnesius

FIGURE 60C AND PLATE 16C

Liver Malate Dehydrogenase patterns in

1. M. cephalus
2. L. parsia
3. V. cunnesius

FIGURE 60D AND PLATE 16D

Liver Octanol Dehydrogenase patterns in

1. M. cephalus
2. L. parsia
3. V. cunnesius

FIG. 60

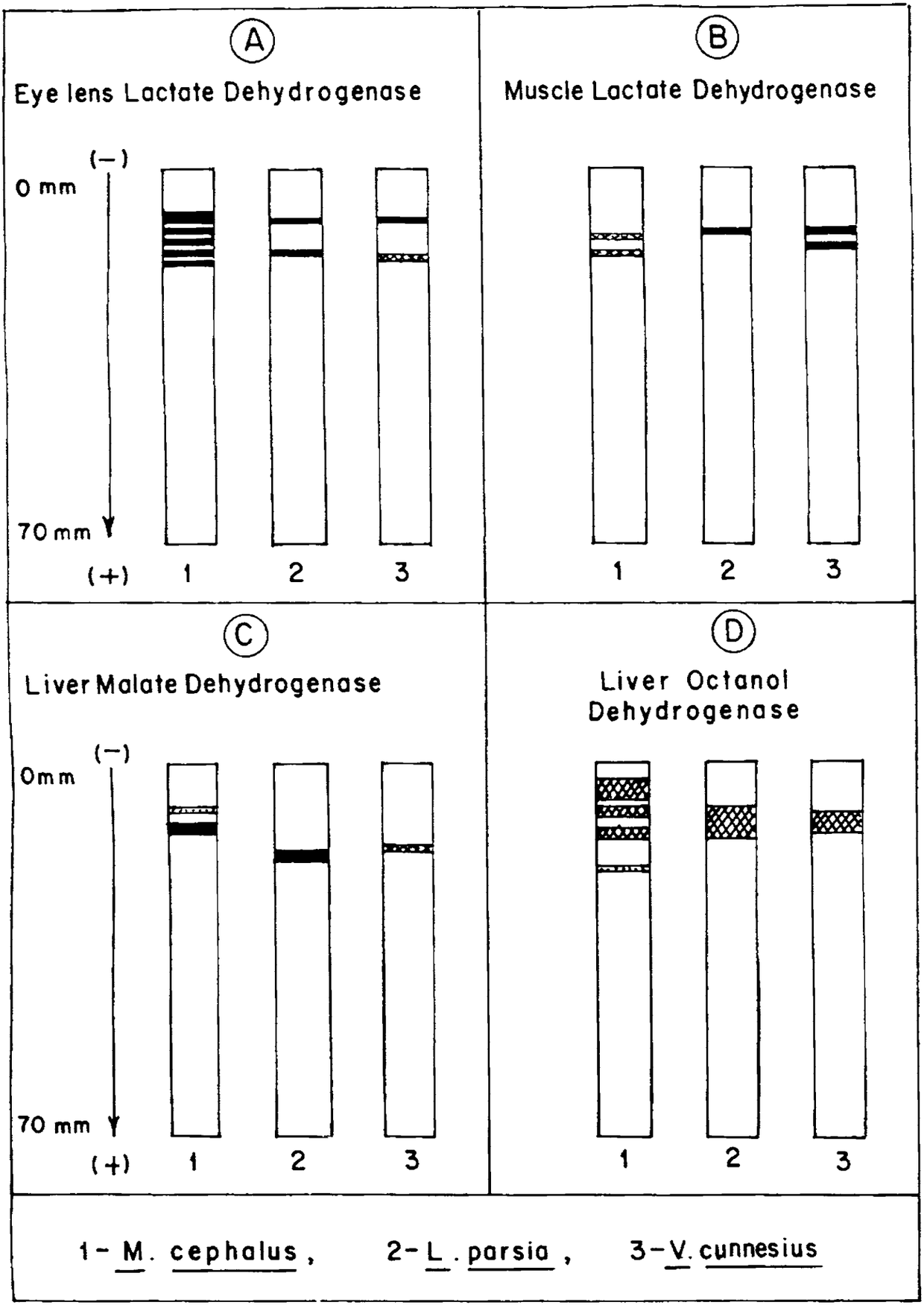
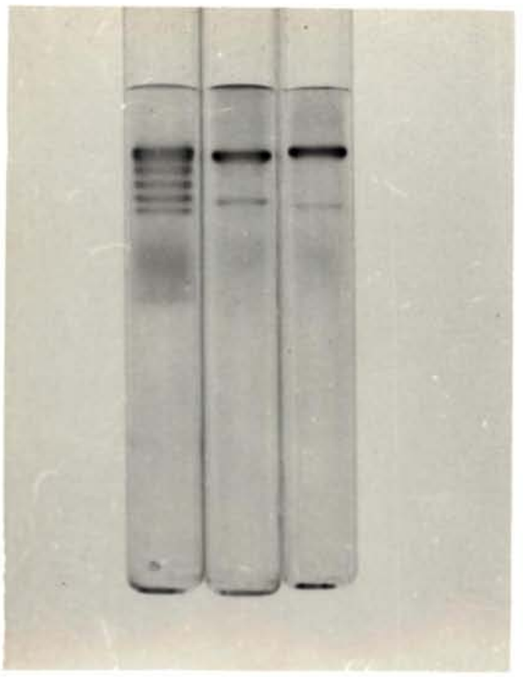

A
Eye lens Lactate Dehydrogenase
(−)
0 mm
70 mm
(+)
1
2
3
B
Muscle Lactate Dehydrogenase
1
2
3
C
Liver Malate Dehydrogenase
(−)
0mm
70 mm
(+)
1
2
3
D
Liver Octanol Dehydrogenase
1
2
3
1- M. cephalus , 2- L. parsia , 3- V. cunnesius

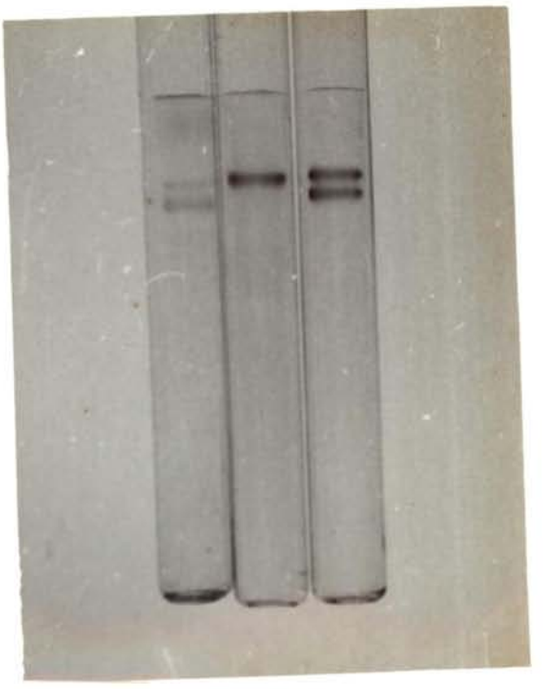
PLATE 16

(A) EYE LENS - LDH



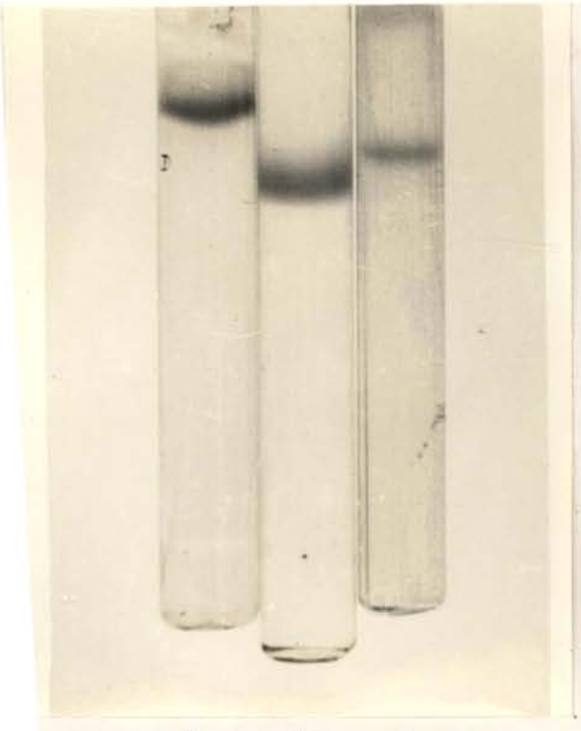
1 2 3

(B) MUSCLE - LDH



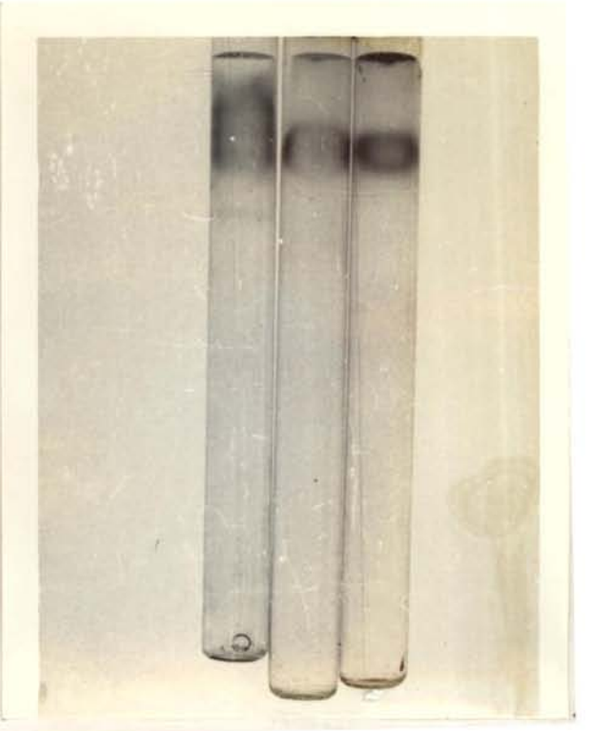
1 2 3

(C) LIVER - MDH



1 2 3

(D) LIVER - ODH



1 2 3

FIGURE 61A AND PLATE 17A

Eye lens Protein patterns in

1. M. cephalus
2. L. parsia
3. V. cunnesius

FIGURE 61B AND PLATE 17B

Muscle Protein patterns in

1. M. cephalus
2. L. parsia
3. V. cunnesius

FIG. 61

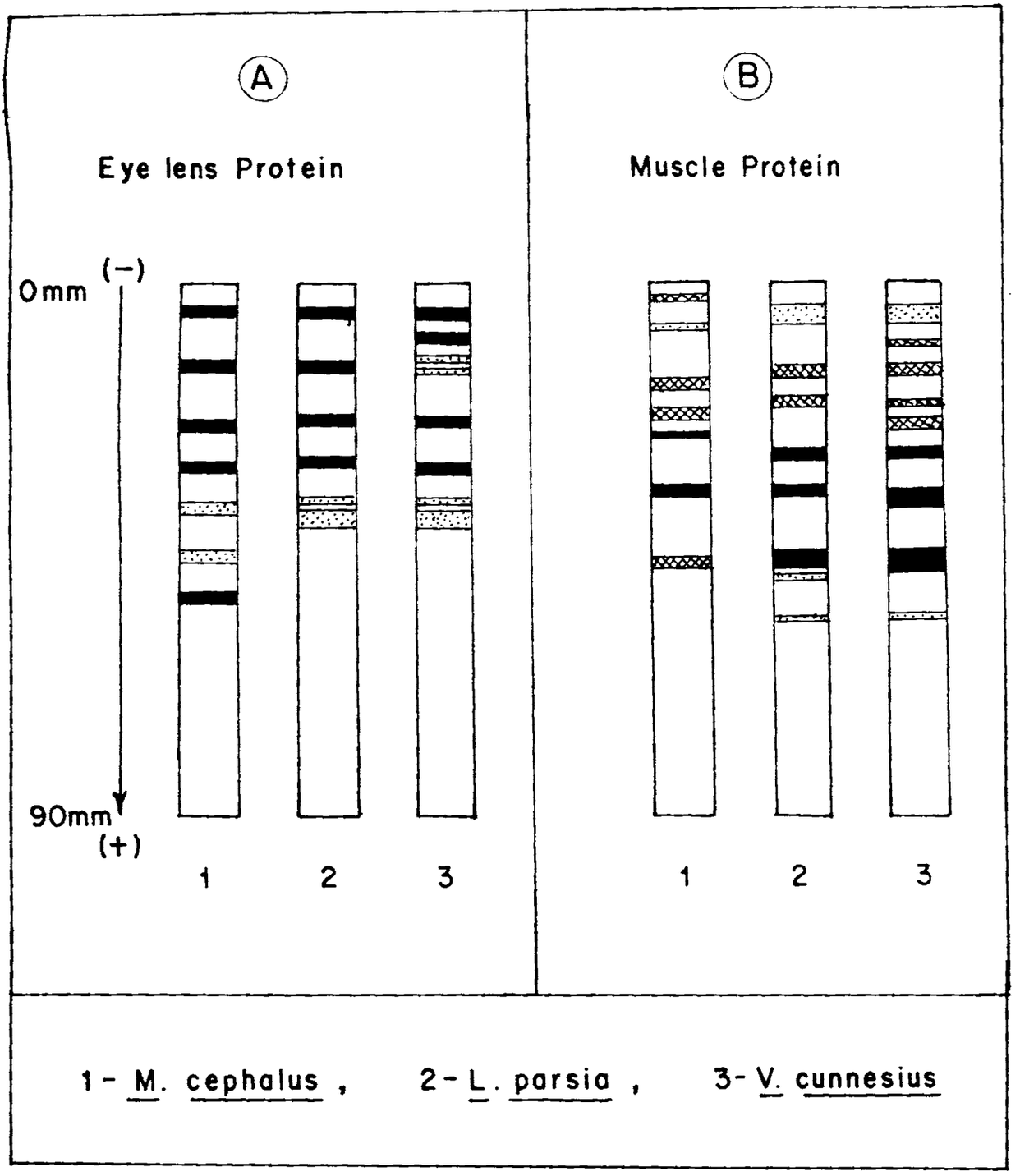
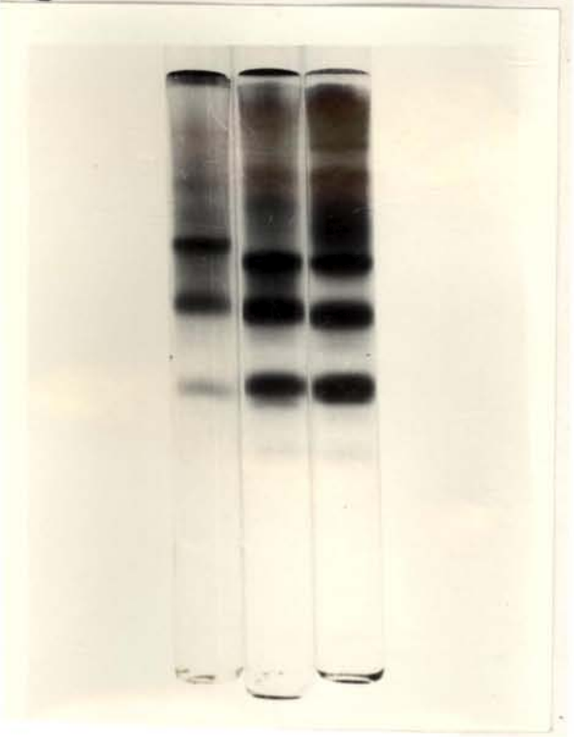

A
Eye lens Protein
0mm
(−)
90mm
(+)
1
2
3
B
Muscle Protein
1
2
3
1- M. cephalus , 2- L. parsia , 3- V. cunnesius

PLATE 17

(A) EYE LENS - PRO

1 2 3

(B) MUSCLE - PRO



1 2 3

FIGURE 62A AND PLATE 18A

Eye lens Pyrroline Dehydrogenase patterns in

1. M. cephalus
2. L. parsia
3. V. cunnesius

FIGURE 62B AND PLATE 18B

Liver Sorbitol Dehydrogenase patterns in

1. M. cephalus
2. L. parsia
3. V. cunnesius

FIGURE 62C

Liver Tetrazolium Oxidase patterns in

1. M. cephalus
2. L. parsia
3. V. cunnesius

FIG. 62

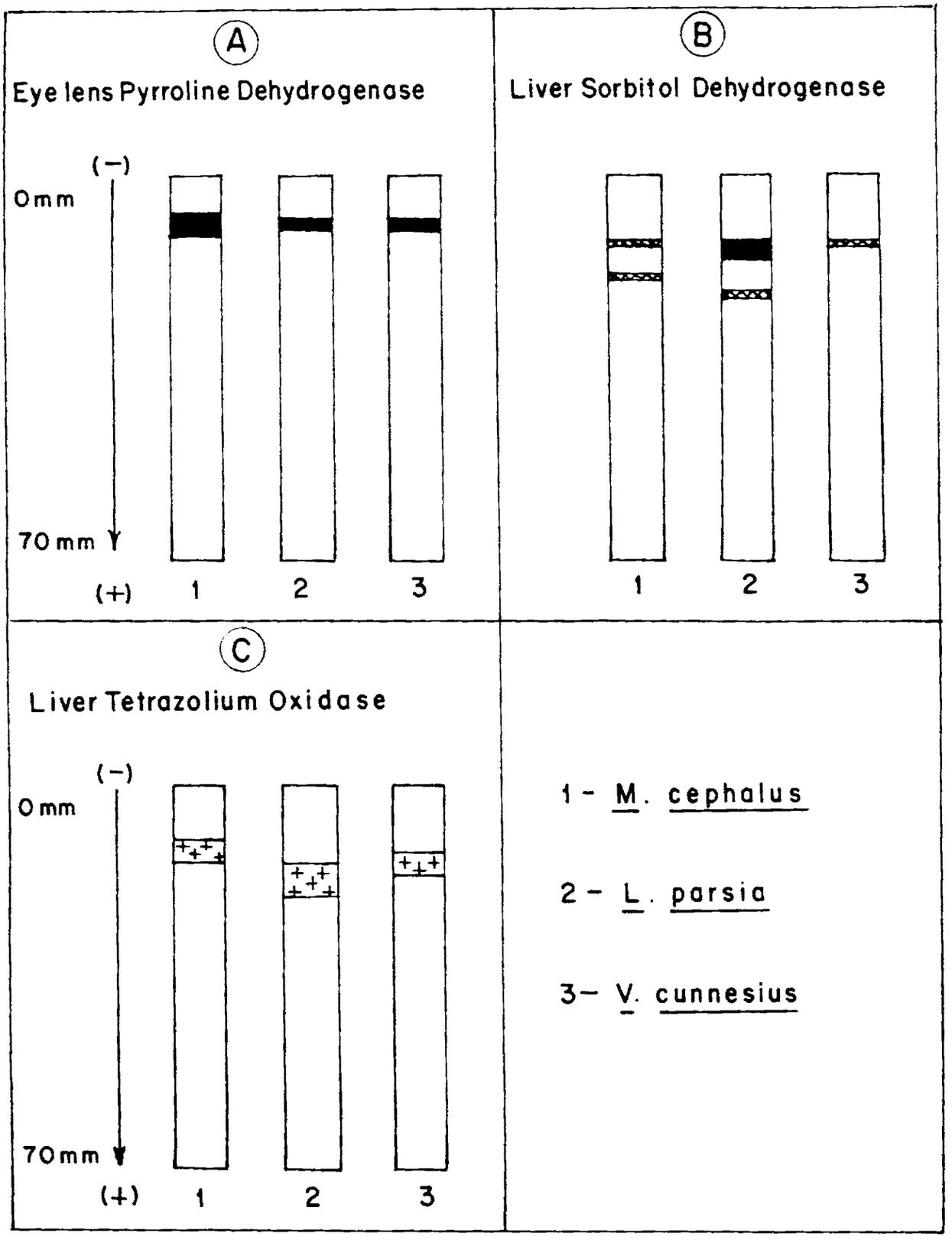
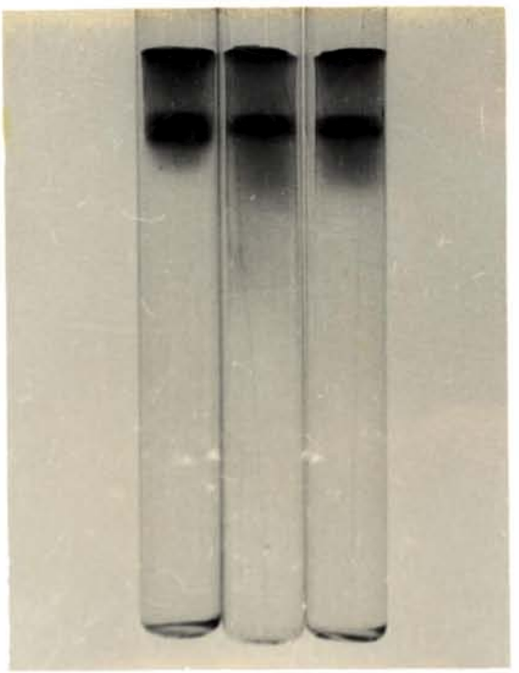

A
Eye lens Pyrroline Dehydrogenase
(−)
0mm
70 mm
(+)
1
2
3
B
Liver Sorbitol Dehydrogenase
1
2
3
C
Liver Tetrazolium Oxidase
(−)
0mm
70mm
(+)
1
2
3
1 - M. cephalus
2 - L. parsia
3 - V. cunnesius

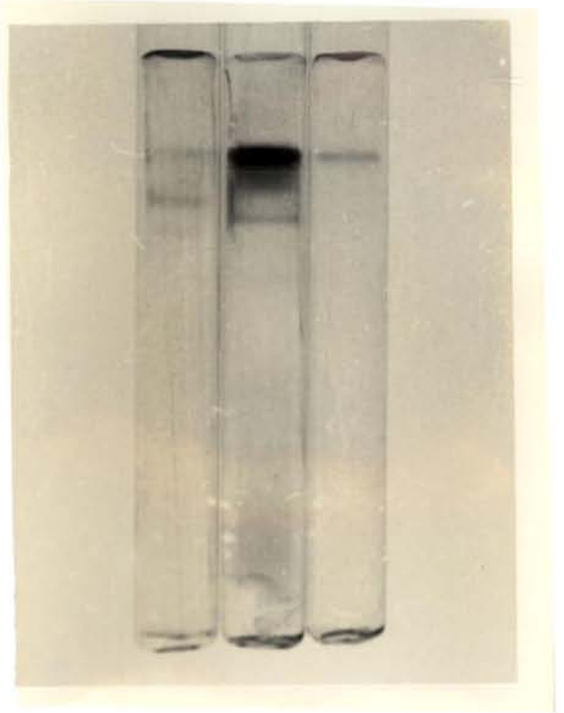
PLATE 18

(A) EYE LENS - PDH



1 2 3

(B) LIVER - SDH



1 2 3

TABLE 18*

A Comparative Details of Electrophoretic Characteristics of Different Enzymes / Proteins in Mugil cephalus (MC), Liza parsia (LP) and Valamugil cunnesius (VC).

Sl. No.	Enzymes	Tissues	Species	Relative Electrophoretic Mobility (mm), Intensity (x) and colourless (C) of different Bands														Total No. of Bands
				1		2		3		4		5		6		7		
1	ACP	L	MC	2-3	3x	7-9	1x	10-11	3x	13	1x	22-30	2x	33-36	2x			6
			LP	4-6	1x	10	1x	12	1x	18-19	1x	20-21	1x	23-27	2x	30-33	1x	7
			VC	7-8	1x	10-11	1x	14-15	1x									3
2	ADH	L	MC	3-4	2x	5-7	2x	8-11	2x	13-14	1x							4
			LP	9-15	2x	16-17	1x											2
			VC	10-17	3x													1
3	ADO	L	MC	10-12	2x													1
			LP	11-12	3x	14-15	2x											2
			VC	10-11	2x	13-14	2x											2
4	ALD	L	MC	9-10	2x													1
			LP	9-12	3x													1
			VC	8-12	3x													1
5	AKP	L	MC	20-22	2x													1
			LP	20-21	1x													1
			VC	20-22	3x													1
6	EST	H	MC	14-17	3x	18-24	3x											2
			LP	14-16	3x	19-22	3x											2
			VC	11-13	3x	14-17	3x											2
7	GDH	E	MC	8-11	1x	14-16	1x											2
			LP	8-11	1x	14-17	1x											2
			VC	8-11	1x													1
8	GPD	L	MC	21-23	1x													1
			LP	0-4	2x	6-8	2x	10-12	2x									3
			VC	8-11	3x													1
9	LDH (E)	E	MC	8-10	3x	11-12	3x	13-14	3x	15-16	3x	17-18	3x					5
			LP	9-10	3x	15-16	3x											2
			VC	9-10	3x	16-17	2x											2

* Refer Page xvi for explanation of abbreviations

TABLE 18* Continued.

Sl. No.	Enzymes	Tissues	Species	Relative Electrophoretic Mobility (mm), Intensity (x) and Colourless (C) of different Bands.									Total No. of Bands
				1	2	3	4	5	6	7	8	9	
10	LDH(M)	M	MC	12-13 2x	15-16 2x								2
			LP	11-12 3x									1
			VC	11-12 3x	14-15 3x								2
11	MDH	L	MC	8-9 1x	11-13 3x								2
			LP	16-18 3x									1
			VC	15-16 2x									1
12	ODH	L	MC	3-7 2x	8-10 2x	12-14 2x	19-20 1x						4
			LP	8-14 2x									1
			VC	9-13 2x									1
13	PRO(E)	E	MC	4-6 3x	13-15 3x	23-25 3x	30-32 3x	37-39 1x	45-49 1x	52-54 3x			7
			LP	4-6 3x	13-15 3x	22-24 3x	29-31 3x	36-37 1x	39-41 1x				6
			VC	4-6 3x	8-10 3x	12-13 1x	14-15 1x	22-24 3x	30-32 3x	36-37 1x	38-41 1x		8
14	PRO(M)	M	MC	1-2 2x	7-8 1x	16-18 2x	21-23 2x	25-26 3x	34-36 3x	46-48 2x			7
			LP	4-7 1x	14-16 2x	19-21 2x	28-30 3x	34-36 3x	45-48 3x	49-50 1x	56-57 1x		8
			VC	4-7 1x	10-11 2x	14-16 2x	20-21 2x	23-25 2x	28-30 3x	35-38 3x	45-49 3x	56-57 1x	9
15	PDH	E	MC	7-11 3x									1
			LP	8-10 3x									1
			VC	8-10 3x									1
16	SDH	L	MC	12-13 2x	18-19 2x								2
			LP	12-15 3x	21-22 2x								2
			VC	12-13 2x									1
17	TZO	L	MC	10-14 C									1
			LP	14-20 C									1
			VC	12-16 C									1

* Refer Page xvi for explanation of abbreviations

species 3 and 1, 2; eye lens LDH between species 1 and 2, 3; muscle LDH between species 2 and 1, 3; MDH between species 2 and 1, 3; ODH between species 1 and 2, 3; eye lens proteins (PRO (E)) between species 1 and 2, 3; muscle protein (PRO (M)) between species 1 and 2, 3; eye lens PDH between species 1 and 2, 3; TZO between species 2 and 1, 3. The species specific differences here are again due to differences in the number of bands on their electrophoretic position or both. The third group patterns are showing more resemblance than differences. The species thus showed resemblances with the corresponding protein patterns are ADH, ADO, ALD, LDH(E), ODH, PRO(M) and PDH between L. parsia and V. cunnesius (FIG. 58B-D, 60A, D, 61B, 62A; PLT. 14B-D, 16A, D, 17B, 18A; TBL. 18). Similar resemblances were observable between M. cephalus and L. parsia for patterns such as AKP, EST and GDH (FIG. 59A-C; PLT. 15A-C; TBL. 18).

4.3 ONTOGENETIC VARIATIONS

During the standardization of electrophoretic patterns of different proteins, including different enzymes, in M. cephalus, certain variations in the protein patterns were observed in different size groups (FIG. 63-67; PLT. 19-21B). The details of size groups, tissue tested, relative electrophoretic mobility and intensity of different bands observed are given in Table 19. A comparison of these patterns indicated presence of non-genetic form of variations, apparently related to size group differences of the specimens tested. Figure 66, Plate 21A and Table 19 show that Group III specimens upto about 3 cm were having only a few protein fractions in their eyes whereas Group I and II specimens above 9.5 cms had several additional protein fractions. There were also differences in the number of protein fractions and their staining intensity between specimens belonging to Groups I and II. Similar differences but at lesser degree level were also noticed in the patterns of muscle proteins (FIG. 67; PLT. 21B; TBL. 19). Here smaller Groups III and II specimens upto 10 cms showed almost comparable number of protein fractions with that of Group I specimens of 28.5 cm. However, the protein fractions particularly in Group III had all bands stained very lightly indicating lesser amount of

FIGURE 63 AND PLATE 19

Liver Alkaline Phosphatase patterns in different size groups of M. cephalus

Group	**I**	:	31.0 to 32.5 cm
Group	**II**	:	15.5 to 19.5 cm
Group	**III**	:	15.5 to 17.0 cm

FIG. 63

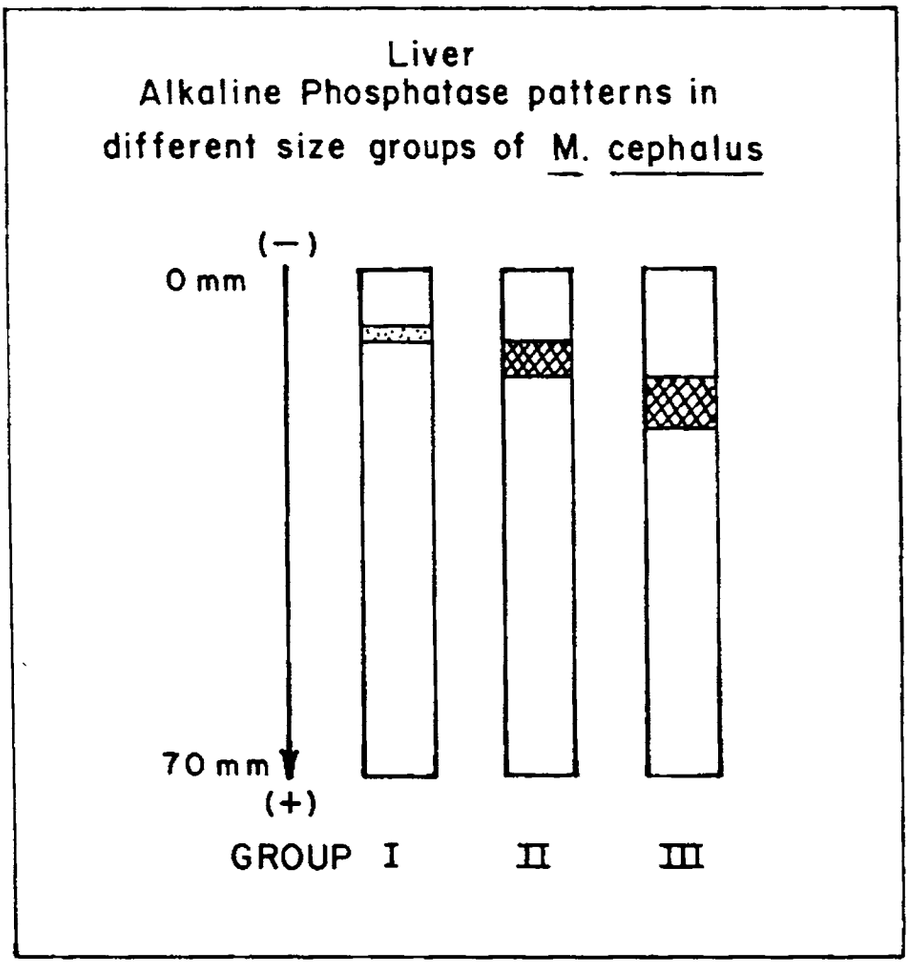
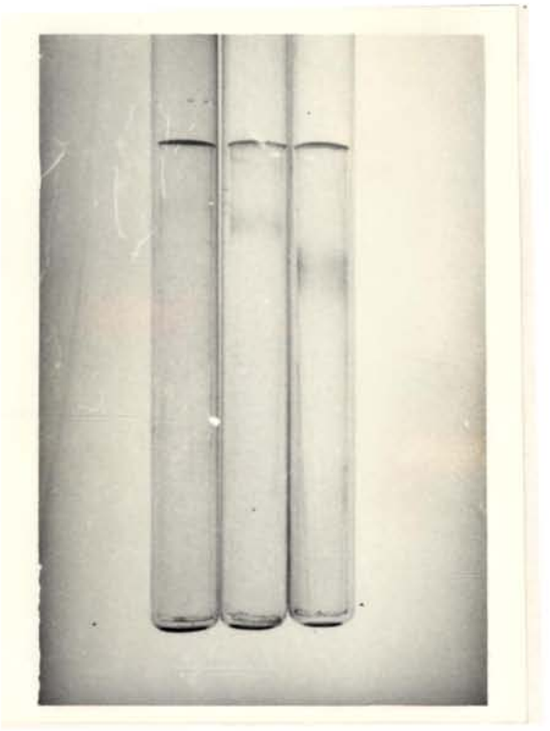

Liver
Alkaline Phosphatase patterns in
different size groups of M. cephalus
(−)
0 mm
70 mm
(+)
GROUP I
II
III

PLATE 19

LIVER – AKP



GROUP I II III

FIGURE 64 AND PLATE 20A

Eye lens Lactate Dehydrogenase patterns in different size groups of M. cephalus

Group	**I**	:	28.5 cm
Group	**II**	:	9.5 to 10.0 cm
Group	**III**	:	2.6 to 3.1 cm

FIGURE 65 AND PLATE 20B

Muscle Lactate Dehydrogenase patterns in different size groups of M. cephalus

Group	**I**	:	28.5 cm
Group	**II**	:	9.5 to 10.0 cm
Group	**III**	:	1: 3.1 cm
			2: 2.6 cm

FIG. 64

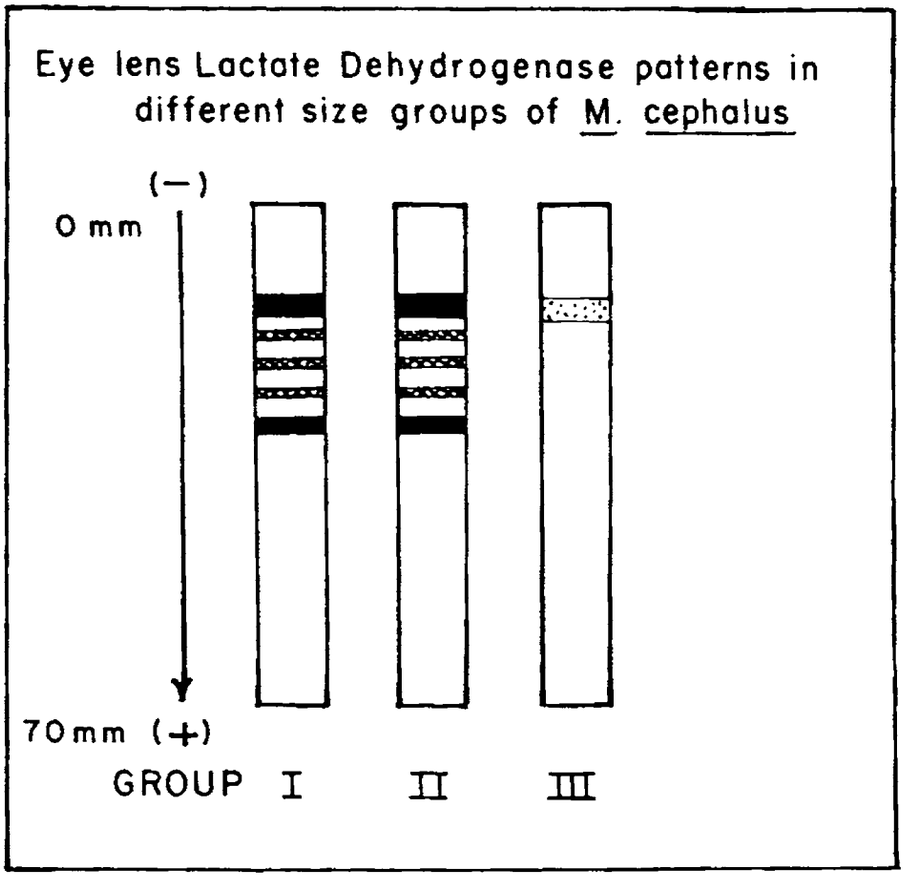

Eye lens Lactate Dehydrogenase patterns in different size groups of M. cephalus
(−)
0 mm
70mm (+)
GROUP I II III

FIG. 65

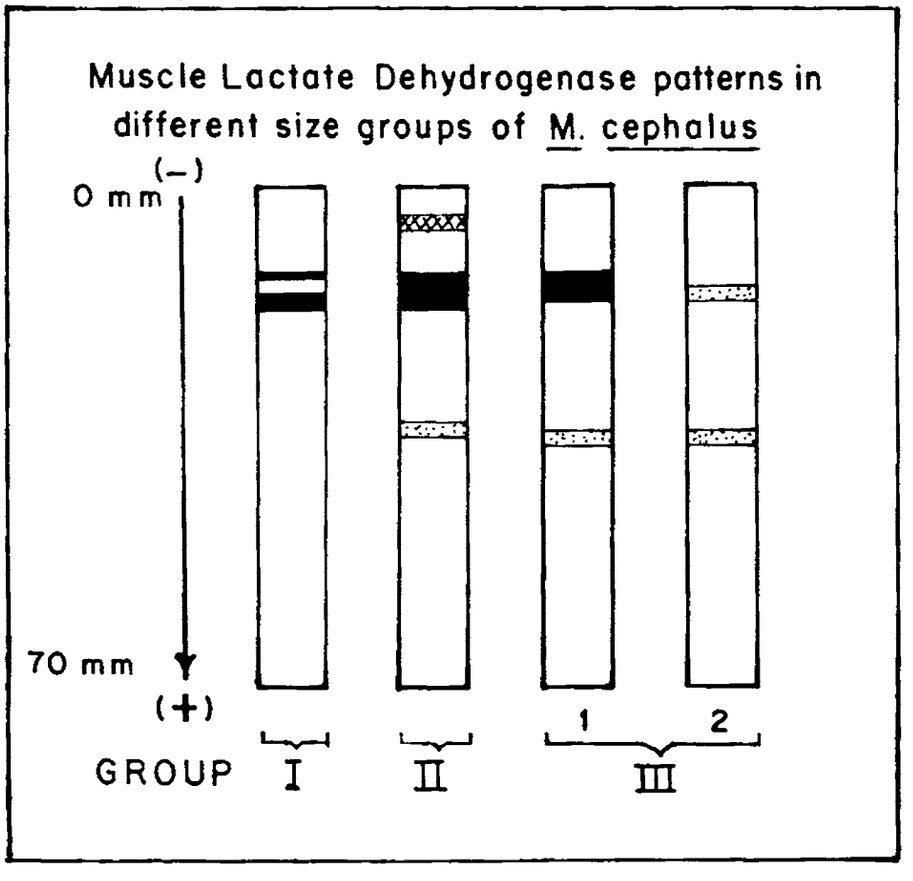

Muscle Lactate Dehydrogenase patterns in different size groups of M. cephalus
(−)
0 mm
70 mm
(+)
1
2
GROUP I II III

PLATE 20

(A) EYE LENS - LDH

(B) MUSCLE - LDH

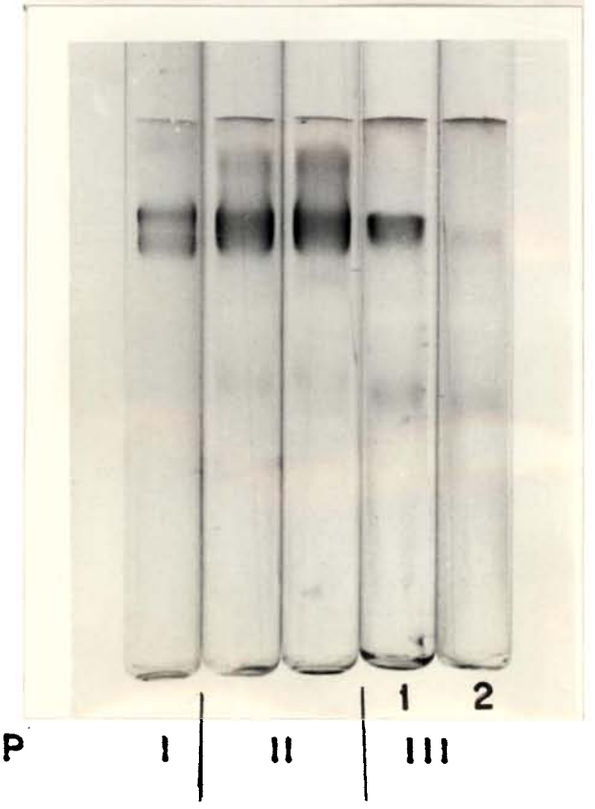


FIGURE 66 AND PLATE 21A

Eye lens Protein patterns in different size groups of M. cephalus

Group	**I**	:	28.5 cm
Group	**II**	:	9.5 to 10.0 cm
Group	**III**	:	2.6 to 3.1 cm

FIGURE 67 AND PLATE 21B

Muscle Protein patterns in different size groups of M. cephalus

Group	**I**	:	28.5 cm
Group	**II**	: 1:	10.0 cm
		2:	9.5 cm
Group	**III**	: 1:	3.1 cm
		2:	2.6 cm

FIG. 66

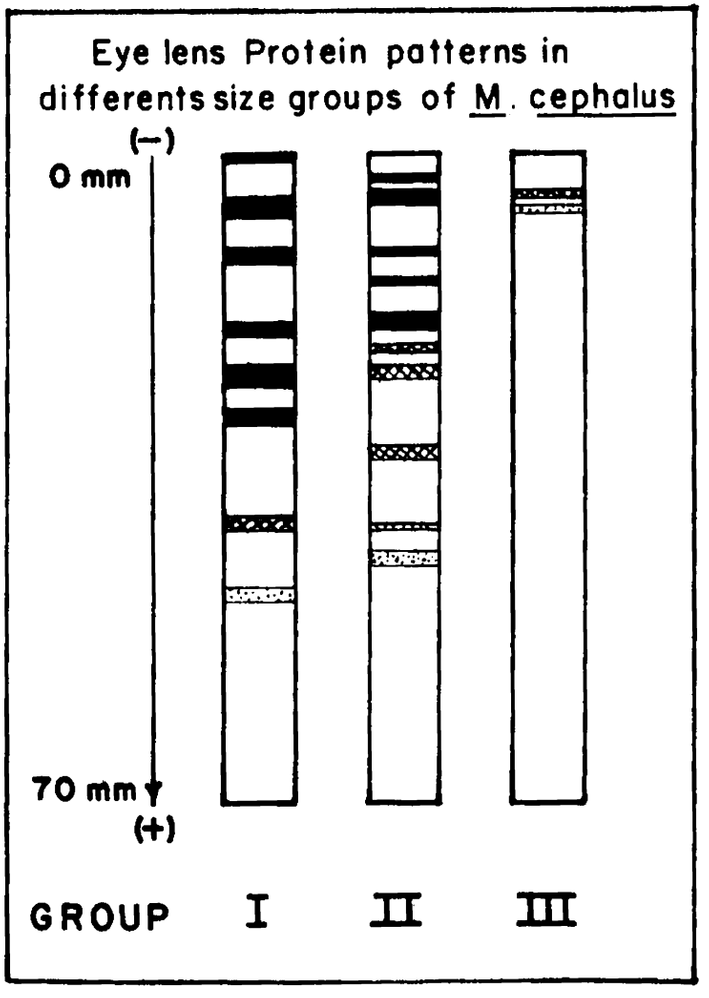

Eye lens Protein patterns in
differents size groups of M. cephalus
(−)
0 mm
70 mm
(+)
GROUP
I
II
III

FIG. 67

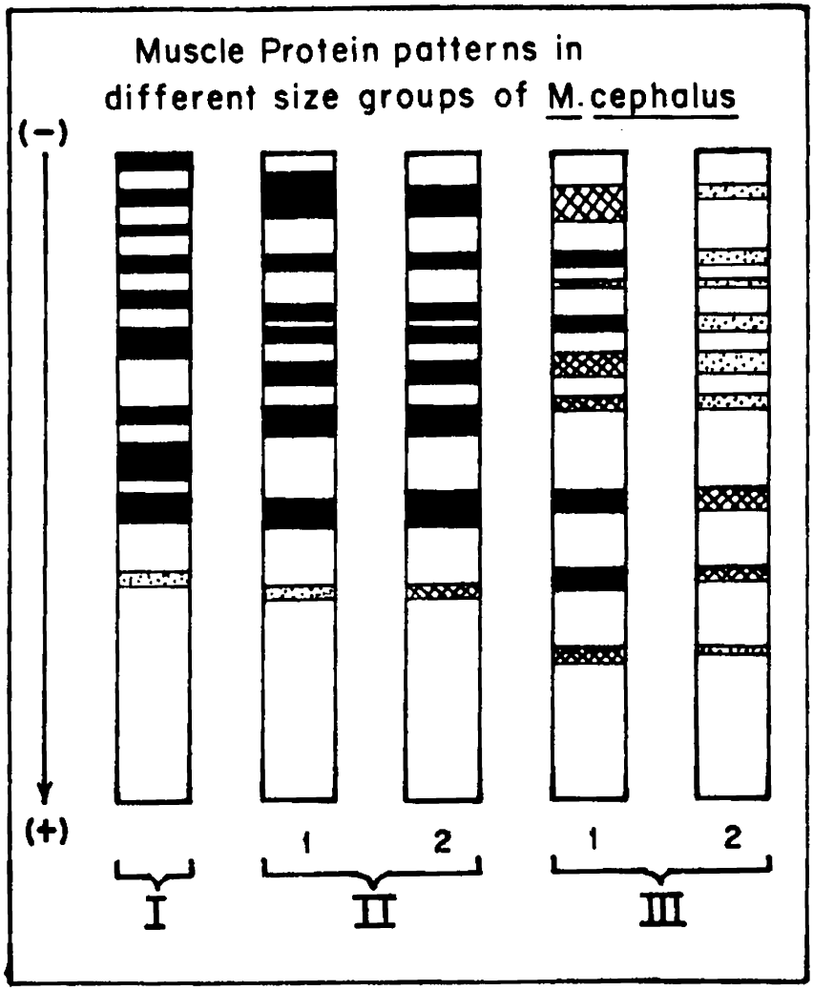

Muscle Protein patterns in
different size groups of M. cephalus
(−)
(+)
1
2
1
2
I
II
III

PLATE 21

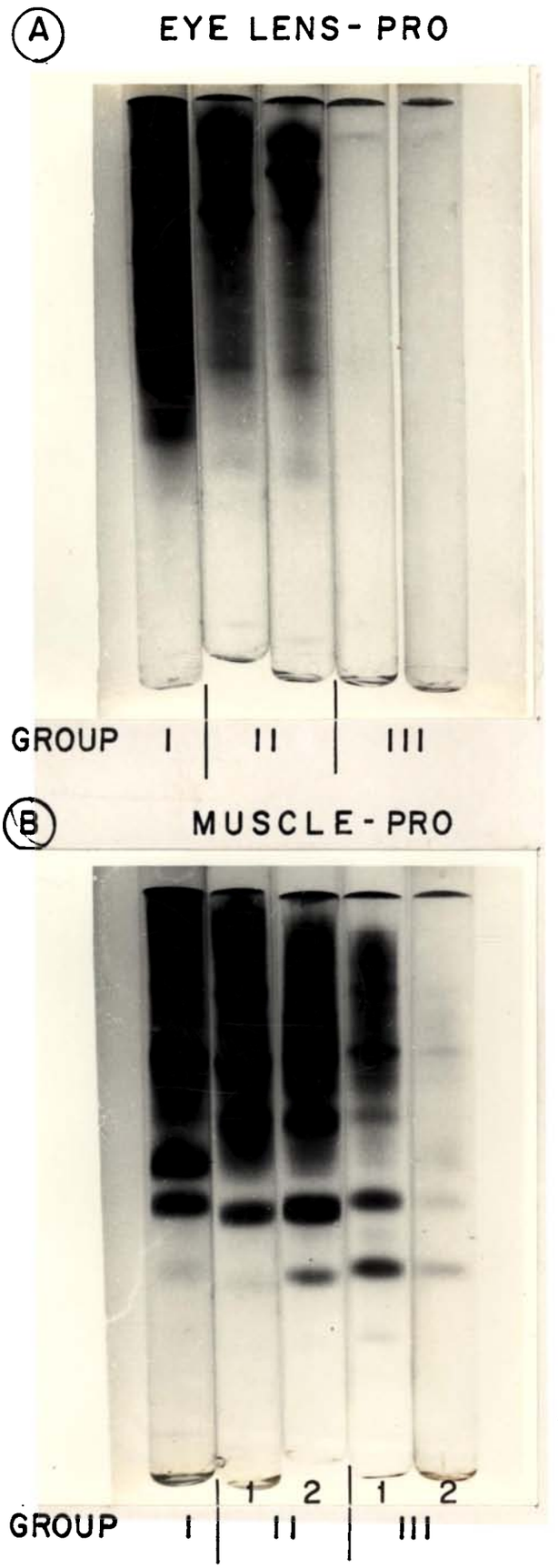


TABLE 19*

Ontogenetic Variations in Different Size Groups of Mugil cephalus L.

Sl. No.	Enzymes	GRP	Size cm	Tissues	Relative Electrophoretic Mobility (mm) and Intensity (x) of Different Bands																						Total No. of Bands
					1		2		3		4		5		6		7		8		9		10				
1	AKP	I	31.0 to 32.5	L	8-10	1x																					1
		II	15.5 to 19.5	L	10-15	2x																					1
		III	15.5 to 17.0	L	15-22	2x																					1
2	LDH(E)	I	28.5	E	13-16	3x	18-19	2x	22-23	2x	26-27	2x	30-32	3x													5
		II	9.5 to 10.0	E	13-16	3x	18-19	2x	22-23	2x	26-27	2x	30-32	3x													5
		III	2.6 to 3.1	E	13-16	1x																					1
3	LDH(M)	I	28.5	M	12-13	3x	15-17	3x																			2
		II	9.5 to 10.0	M	4-6	2x	12-17	3x	33-35	1x																	3
		III(1)	3.1	M	12-16	3x	34-36	1x																			2
		(2)	2.6	M	14-16	1x	34-36	1x																			2
4	PRO(E)	I	28.5	E	0-1	3x	6-9	3x	13-15	3x	23-25	3x	29-32	3x	35-37	3x	50-52	2x	60-62	1x							8
		II	9.5 to 10.0	E	3-4	3x	5-7	3x	13-14	3x	17-18	3x	22-24	3x	26-27	2x	29-31	2x	40-42	2x	51-52	1x	55-57	1x			10
		III	2.6 to 3.1	E	5-6	2x	7-8	1x																			2
5	PRO(M)	I	28.5	M	0-2	3x	5-7	3x	10-11	3x	14-16	3x	19-21	3x	24-28	3x	35-37	3x	40-45	3x	47-51	3x	58-60	1x			10
		II (1)	10.0	M	3-9	3x	14-16	3x	21-23	3x	24-26	3x	29-32	3x	35-39	3x	48-52	3x	60-62	1x							8
		(2)	9.5	M	5-9	3x	14-16	3x	21-23	3x	24-26	3x	29-32	3x	35-39	3x	47-52	3x	60-62	2x							8
		III (1)	3.1	M	5-10	2x	14-16	3x	18-19	3x	23-25	3x	28-31	2x	34-36	2x	47-50	3x	58-61	3x	69-71	2x					9
		(2)	2.6	M	5-7	1x	14-16	1x	18-19	1x	23-25	1x	28-31	1x	34-36	1x	47-50	2x	58-60	2x	69-70	1x					9

* Refer Page xvi for explanation of abbreviations

proteins. There were also differences noticed in the width and position of protein fractions between these three groups.

Ontogenetic type of differences were also observed in the patterns of liver Alkaline Phosphatase, eye lens and muscle LDH enzymes (FIG. 63-65; PLT. 19-20B; TBL. 19). As figure 63 shows though AKP enzyme showed a single band in all three size groups, electrophoretic position and width differed in each group. In the case of eye lens LDH enzyme only group III specimens of 2.6 - 3.1 cm showed strong difference having a single slowest moving, lightly stained band instead of five banded patterns present in groups I and II specimens above 9.5 cm (FIG. 64; PLT. 20A). On the other hand, muscle LDH differences were of lesser degree between these groups (Fig.65; PLT. 20B). One faster moving lightly stained additional band was present in group II and III whereas lightly stained slower moving additional band was also shown in group II. Group I showed two bands instead of one broad band at the comparable gel position in groups II and III specimens.

4.4 GENERAL PROTEINS

4.4.1 EYE LENS PROTEIN (PRO (E))

The eye lens protein zymograms obtained from M. cephalus sample populations collected from Cochin, Madras and Orissa are shown in the Figure 74 and Plate 27. These zymograms were prepared on the basis of comparable electrophoretic patterns of eye lens proteins obtained from individual specimens from each region and differentiating total number of specimens tested into group patterns existing in each region. Thus, the number of zymograms varied from three in Cochin and Orissa and four in Madras. As also observed in the case of muscle proteins, the number, size, intensity and electrophoretic mobility of eye lens protein bands varied between zymogram groups in each region and also between regions. Specific variations in the band patterns at a particular gel area were compared to locate phenotype variations and infer corresponding loci that may be controlling them. Thus five polymorphic loci were located in the eye lens protein in M. cephalus populations tested in the present investigation. The nature of phenotypes in each locus in each

FIGURE 74 AND PLATE 27

Zymogram patterns of Eye lens Protein in M. cephalus populations from Madras and Orissa.

Locus	Alleles		
I	S	=	5-7 mm
	F	=	9-11 mm
II	S	=	13-15 mm
	F	=	18-20 mm
III	S	=	23-25 mm
	F	=	29-31 mm
IV	S	=	34-35 mm
	F	=	36-38 mm
V	S	=	45-48, 46-47 mm
	F	=	51-52, 51-53 mm

Locus	Cochin	Madras	Orissa
I	S	S	S
		SF	
		F	
II	S	S	S
		SF	SF
		F	
III	SF	SF	SF
IV.	SF		
	F	F	F
V	S		
	SF	SF	SF
		F	F

FIG.74

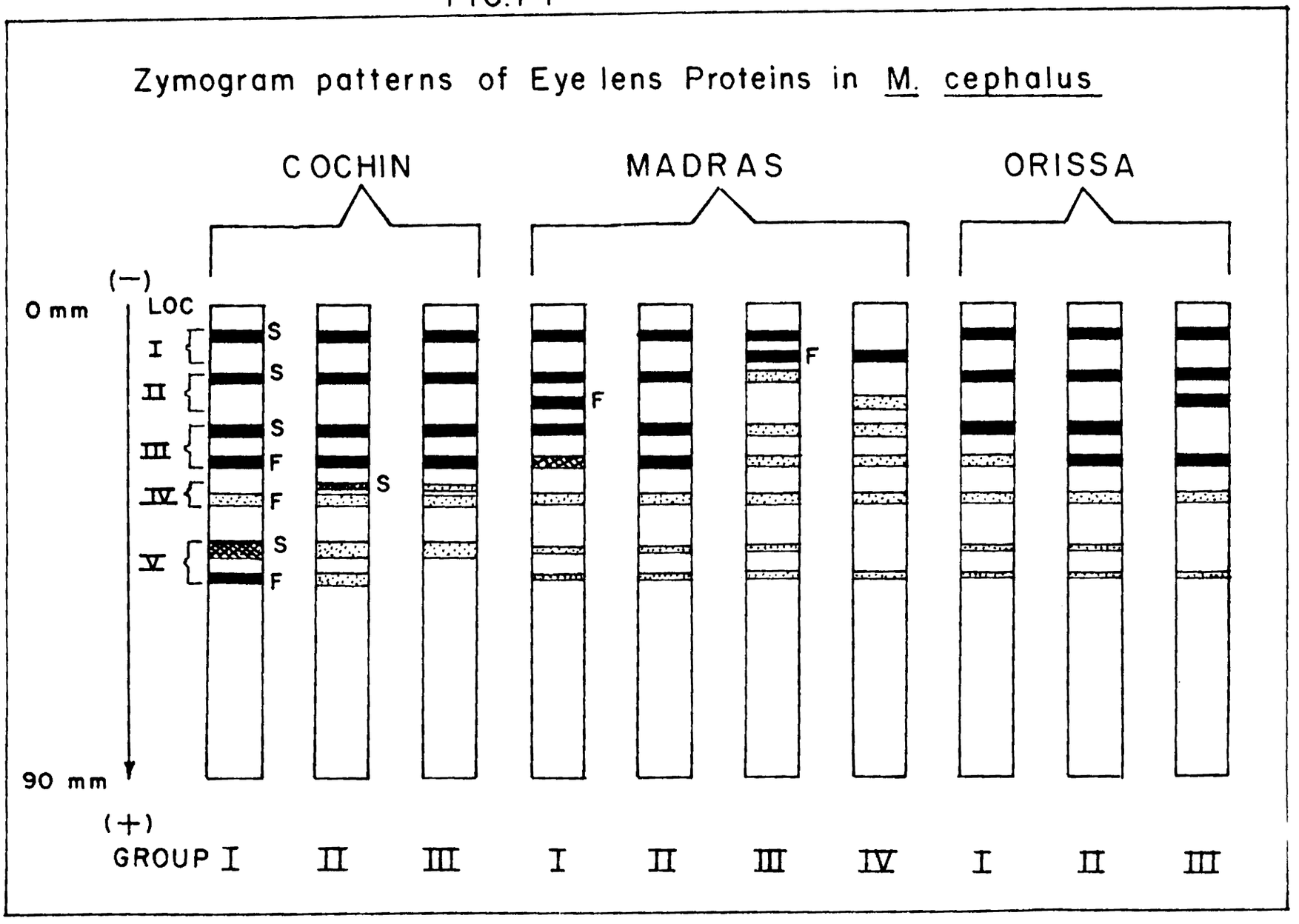
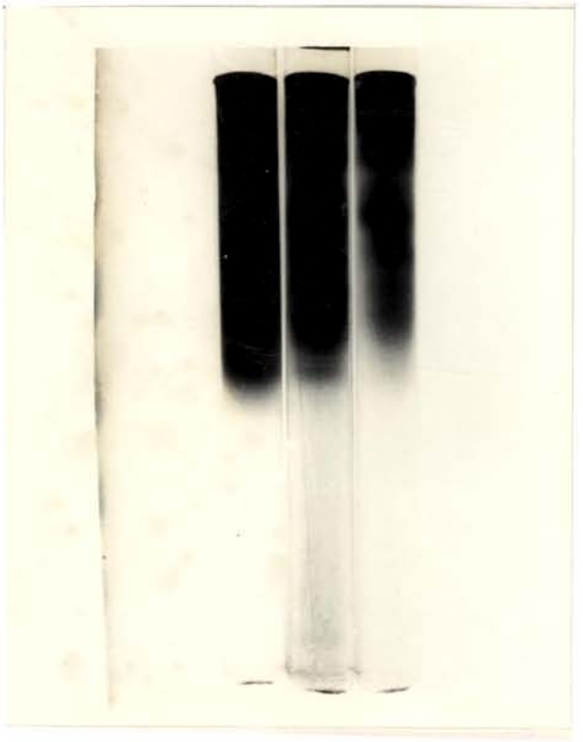

Zymogram patterns of Eye lens Proteins in M. cephalus
COCHIN
MADRAS
ORISSA
(−)
0 mm
LOC
I
II
III
IV
V
S
S
S
F
F
S
S
F
F
S
F
90 mm
(+)
GROUP I II III I II III IV I II III

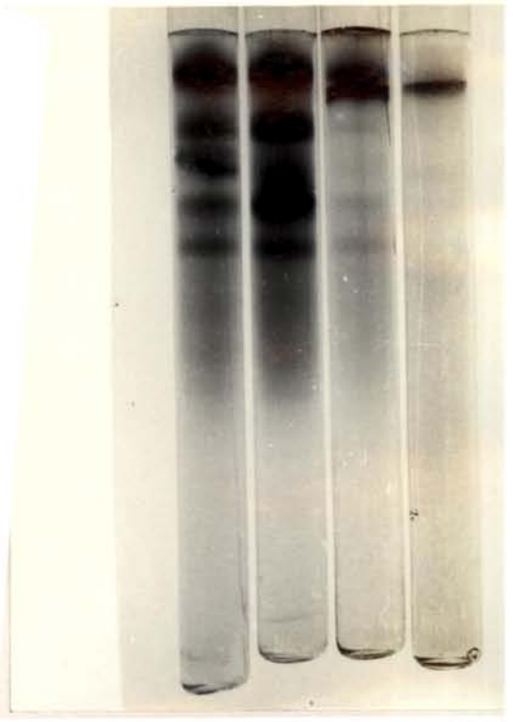
PLATE 27

EYE LENS-PRO

COCHIN



MADRAS



ORISSA

population is described below. In each region each of the five locus showed a genotype either in the form of a homozygote or heterozygote/or both.

Locus I :

The first locus in Cochin and Orissa showed a single banded monomorphic **S** phenotype (5-7 mm). It showed three polymorphic phenotypes **S** (5-7 mm), **SF** (5-7/9-11 mm) and **F** (9-11 mm) in Madras.

Locus II :

The second locus in Cochin showed again only a single banded monomorphic **S** phenotype (13-15 mm). It showed three polymorphic phenotypes **S** (13-15 mm), **SF** (13-15/18-20 mm) and **F** (18-20 mm) in Madras. The staining intensity of **S** phenotype (13-15 mm) and **F** phenotype (18-20 mm) was much less in certain specimens in Madras than for other regions for the same bands. Orissa indicated only two polymorphic phenotypes **S** (13-15 mm) and **SF** phenotype (13-15/18-20 mm).

Locus III :

The third locus in Cochin and Madras showed only two banded **SF** phenotype with band position (23-25/29-31 mm) whereas in Orissa it showed two polymorphic phenotypes **SF** and **F** with band positions (23-25/29-31 mm) and (29-31 mm) respectively. Most specimens in Madras showed **SF** phenotype as much lightly stained.

Locus IV :

The fourth locus in Cochin showed two polymorphic phenotypes, **SF** and **F** having band positions (34-35/36-38 mm) and (36-38 mm) respectively. In Madras and Orissa it showed only a single banded **F** phenotype with band position (36-38 mm). The fourth locus phenotype bands in all the region were lightly stained.

Locus V :

The fifth locus in Cochin indicated two polymorphic phenotypes **S** and **SF** with band positions (45-48 mm) and (45-48/51-53 mm) respectively. In Madras and Orissa also it showed two polymorphic phenotypes **SF** and **F** having band positions (46-47/51-52 mm) and (51-52) respectively. Again all bands except the **F** band in few specimens in Cochin were lightly stained (FIG. 74; PLT. 27 mm).

Thus the polymorphism at each of the five eye lens protein loci in M. cephalus is produced by two codominant alleles. The general comparison of eye lens protein phenotype patterns at five assumed loci among three regions revealed certain important genetic characteristics at each locus. The first locus of Cochin and Orissa were having only **S** homozygote. The first polymorphic locus in Madras showed both **S** and **F** homozygotes and **SF** heterozygote. Though the second locus in Cochin showed only the **S** homozygote, Madras showed **S** and **F** homozygotes as well as **SF** heterozygote. However, Orissa had only **S** homozygote and **SF** heterozygote. On the other hand, the third locus in Cochin and Madras was similar having **SF** heterozygote whereas Orissa had an additional single banded **F** homozygote. The fourth locus was similar in Madras and Orissa having only the **F** homozygote, whereas in Cochin, it had both **F** homozygote as well as **SF** heterozygote. The fifth locus was polymorphic in all the three regions. However, Cochin alone had the **S** homozygote whereas Madras and Orissa alone had **F** homozygote. Thus out of 15 expected phenotypes a total of 13 specific phenotypes were observed at PRO (E) I to V loci in M. cephalus populations tested in the present investigation (TBL. 23).

The allelic frequencies calculated from the observed genotypes at all these five eye lens protein loci (PRO (E) I-V) in M. cephalus populations from three regions are presented in Table 21. The allelic frequencies at many of these five loci differed considerably between M. cephalus populations. Though in PRO (E) I locus **S** allele showed cent percent frequency in Cochin

and Orissa, it showed only 0.90 frequency in Madras while 0.10 frequency was shared by a second allele **F**. PRO (E) II locus also showed similar considerable allelic difference between regions having polymorphism at this locus in Madras (**S** = 0.90, **F** = 0.10) and Orissa (**S** = 0.87, **F** = 0.13) whereas monomorphism in Cochin (**S** = 1.00). Though allelic frequencies at PRO (E) III were identical in Cochin and Madras (0.50 each), Orissa showed 0.38 and 0.62 frequencies for **S** and **F** allele respectively. The reason for identical frequencies is due to observation of all specimens from these two regions as heterozygotes (TBL. 23). PRO (E) IV locus showed very significant allelic difference between Cochin and other two regions. Because while Cochin population was highly polymorphic (**S** = 0.25, **F** = 0.75), the other two populations were having cent percent **F** allele. Though PRO (E) V locus was highly polymorphic in all three regions the nature of allelic frequency distribution was strikingly different, particularly, between Cochin and Madras and also between Cochin and Orissa. Because **S** was the dominant allele at Cochin with a frequency of 0.62 whereas, allele **F** dominated both Madras (0.52) and Orissa (0.62) regions. The expected genotype frequency distributions at all these loci according to H.W. Law were calculated and the values are given in the Table 23. PRO (E) loci III and V deviated significantly at $P > 0.001$ level due to excess of heterozygotes. PRO (E) I and II showed deviation at $P < 0.01$ level at Madras region only. PRO (E) IV locus also showed deviation at $P < 0.01$ level at Cochin region only.

4.4.2 MUSCLE PROTEIN (PRO (M))

The muscle protein zymograms obtained from sample populations of M. cephalus collected from Cochin, Madras and Orissa are shown in Figure 75 and Plate 28. Following the method of eye lens proteins four zymogram groups in Cochin, three in Madras and one in Orissa were recognized as shown in the Figure 75. An overall comparison of the number, the size and staining intensity of each zymogram group indicated considerable variation within and between regions. A close comparison of these protein band variations between zymogram groups at a particular gel area indicated that they may be allelic products of a particular locus. Thus a general comparison of varying

FIGURE 75 AND PLATE 28

Zymogram patterns of Muscle Protein in M. cephalus populations from Madras and Orissa.

Locus	Alleles		
I	S	=	1-2 mm
	F	=	9-10 mm
II	S	=	13-14 mm
	F	=	16-17 mm
III	S	=	21-24 mm
	F	=	32-35 mm
IV	S	=	40-43 mm
	F	=	45-48 mm

Locus	Cochin	Madras	Orissa
I		S	
		SF	
	F	F	F
II	S	S	
	SF	SF	SF
III		S	
	SF	SF	SF
IV	S		
		SF	
		F	F

FIG.75

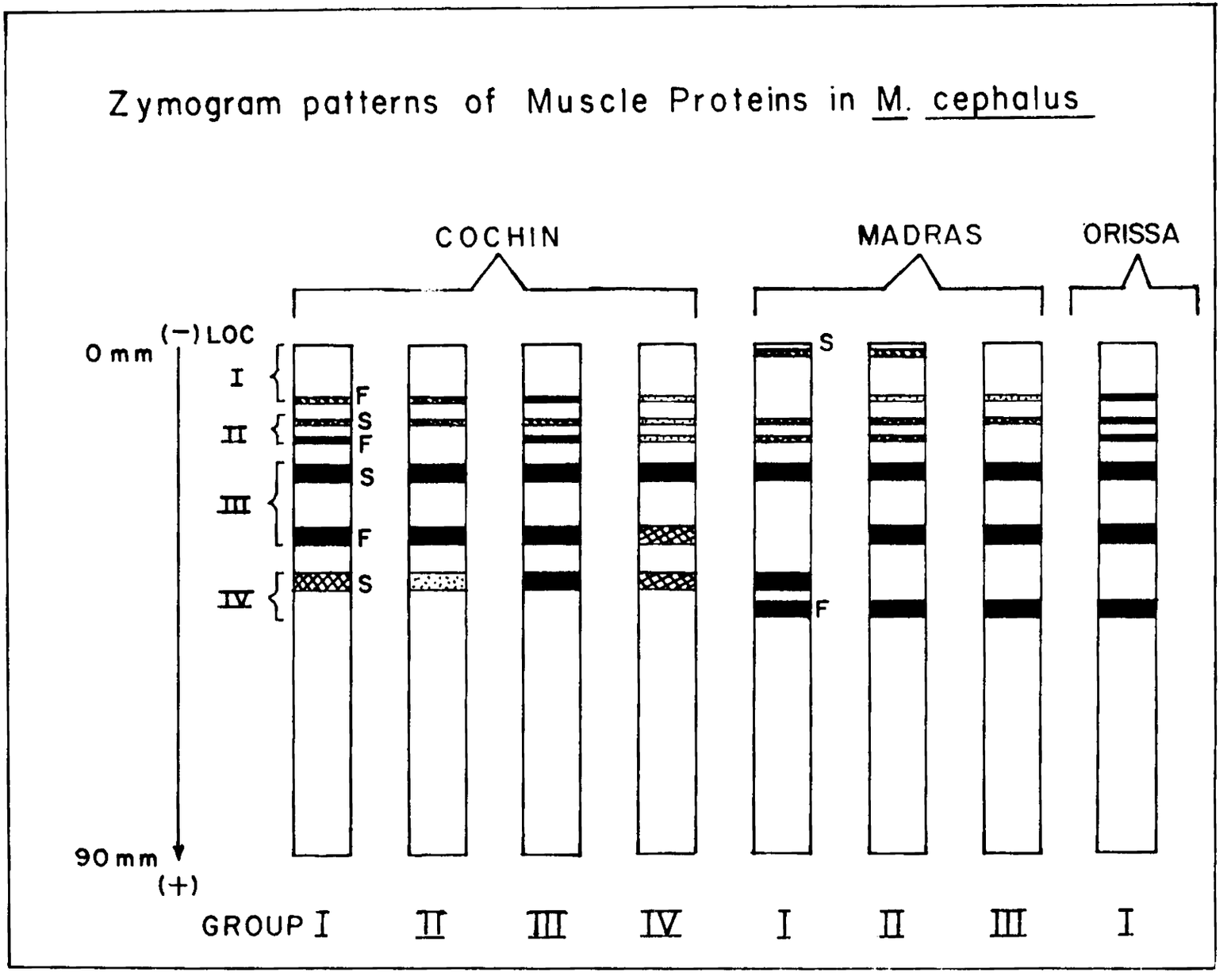
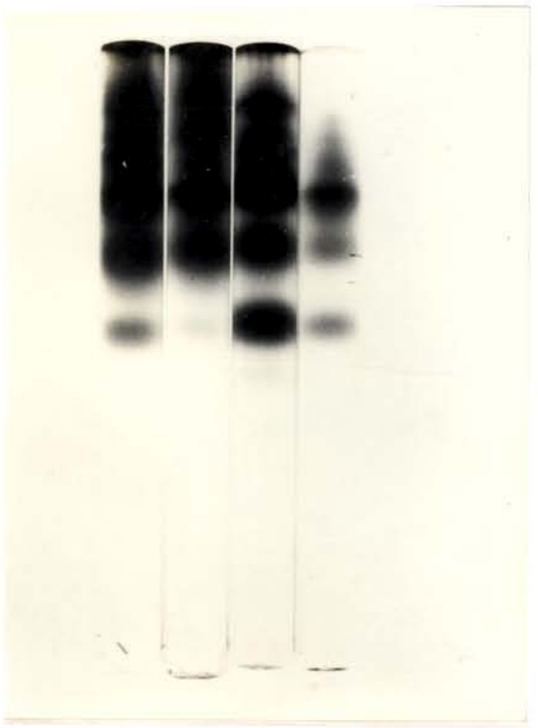

Zymogram patterns of Muscle Proteins in M. cephalus
COCHIN
MADRAS
ORISSA
0 mm
(−) LOC
I
II
III
IV
F
S
F
S
F
S
S
F
90 mm
(+)
GROUP I
II
III
IV
I
II
III
I

PLATE 28

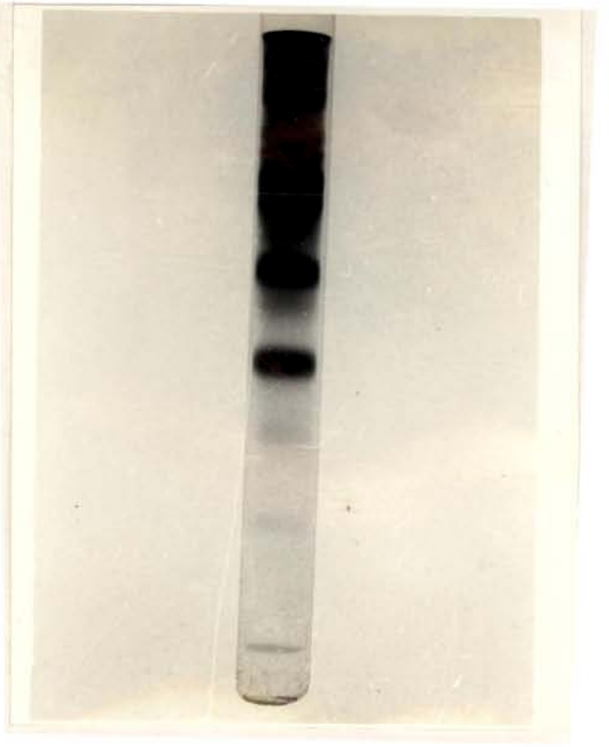
MUSCLE-PRO

COCHIN



MADRAS

ORISSA



and non-varying band groups at a particular gel area for all groups indicated the existence of four polymorphic loci responsible for the expression of all the muscle protein phenotypes observed in the sample populations of M. cephalus tested here. The phenotypes observed at each locus in each population are described below.

Locus I :

The first locus in Cochin and Orissa showed only a single phenotype designated as **F** with band position (9-10 mm). The first locus in Madras showed three different phenotypes namely **S, SF** and **F** with band positions (1-2 mm), (1-2/9-10 mm) and (9-10 mm) respectively (FIG. 75 mm).

Locus II :

The second locus in Cochin and Madras exhibited two variant phenotypes namely **S** and **SF** with band positions (13-14 mm) and (13-14/16-17 mm) respectively. Orissa showed only **SF** phenotype having band position (13-14/16-17 mm) (FIG. 75).

Locus III :

The third locus in Cochin and Orissa produced only a single **SF** phenotype having band positions (21-24/32-35 mm). The third locus in Madras, on the other hand, showed two polymorphic phenotypes, namely **S** and **SF** having band positions (21-24 mm) and (21-24/32-35 mm) respectively (FIG. 75).

Locus IV :

The fourth locus in Cochin produced a single monomorphic **S** phenotype with band position (40-43 mm). The fourth locus in Madras showed two variant phentypes **SF** and **F** with band positions (40-43/45-48 mm) and (45-48 mm) respectively. The fourth locus in Orissa produced a single monomorphic **F** phenotype with band position (45-48 mm). As in the case of eye lens protein, certain muscle protein bands at certain loci were less stained than other band (FIG. 75).

Thus a codominant diallelic polymorphism occured at four muscle protein loci in M. cephalus populations. Assuming the observed phenotype variations are genotype variations, allelic frequencies at all these four muscle protein loci were calculated for each populations and the values are given in Table 21. A comparison of allelic frequencies at each of the four muscle protein loci shows that their values differ considerably between populations. Though Cochin and Orissa showed cent percent **F** allele (F = 1.00) at first locus, it was highly polymorphic at Madras (S = 0.63, F = 0.37). It is also interesting to note that the dominant allele at Madras was **S** as against **F** in other two regions. Allelic frequencies at second locus in all three regions are almost similar. Though third locus showed equal allelic frequencies at Cochin and Orissa (0.50 each), **S** allele dominated with 0.70 frequency at Madras. As all specimens tested from Cochin and Orissa were heterozygotes, the frequencies are found to be equal (TBL. 21). The allelic frequencies at fourth locus was considerably different among three regions. When Cochin showed cent percent **S** allele Orissa showed cent percent **F** allele. On the other hand Madras with frequencies 0.80 for **S** allele and 0.20 for **F** allele showed a different order of allelic frequencies. The values for expected genotype frequency distribution are also presented in Table 23. The expected value for genotype frequency distribution deviated significantly from that of observed at $P > 0.001$ level at loci II and III in all the three regions. It deviated at $P < 0.01$ level at IV locus in Madras.

4.5 ENZYME SYSTEMS

4.5.1 ACID PHOSPHATASE (ACP)

The ACP zymogram patterns obtained in M. cephalus from Cochin, Madras and Orissa are shown in the Figure 68. The zymogram of ACP showed three regions of enzyme activity suggesting the existence of three possible loci (FIG. 68 and PLT. 22). The enzyme activity at third locus was feeble and lightly stained. Hence, third locus was not considered for comparison.

The first locus phenotype (0-2 mm) was similar and monomorphic in Cochin, Madras and Orissa. The region considered as second locus showed

FIGURE 68 AND PLATE 22

Zymogram patterns of Acid Phosphatase in M. cephalus populations from Cochin, Madras and Orissa.

The following genotypes were shown at 1st and IInd loci.

Locus	**I**	: **S**	=	0-2 mm
Locus	**II**	: **S**	=	5-7 mm
		S1	=	8-10 mm
		F	=	12-15 mm
		F1	=	19-21 mm

Locus	Cochin	Madras	Orissa
I	S	S	S
II		SF	SF1
			S1F1
	F	F	F
		FF1	FF1
	F1	F1	F1

FIG. 68

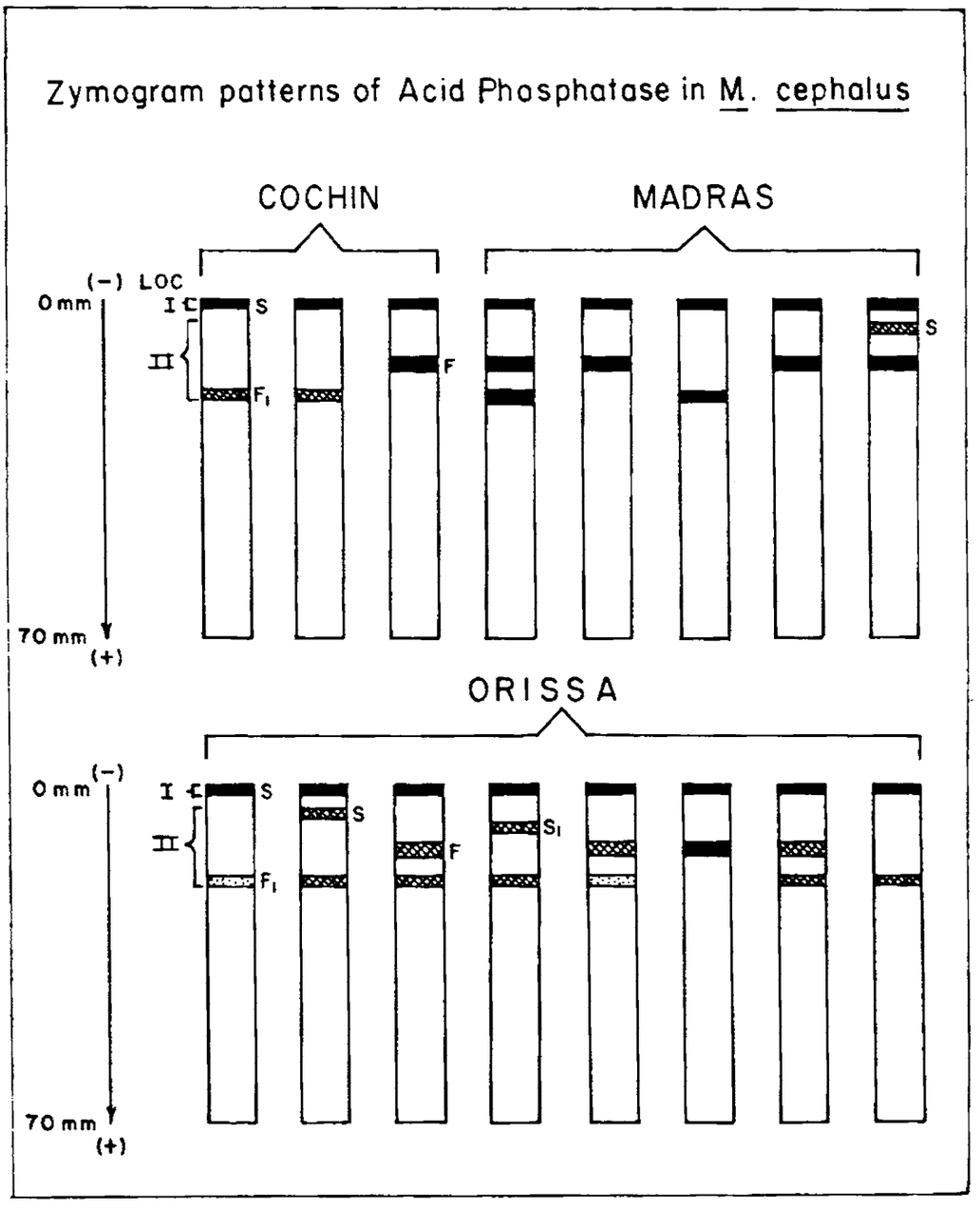
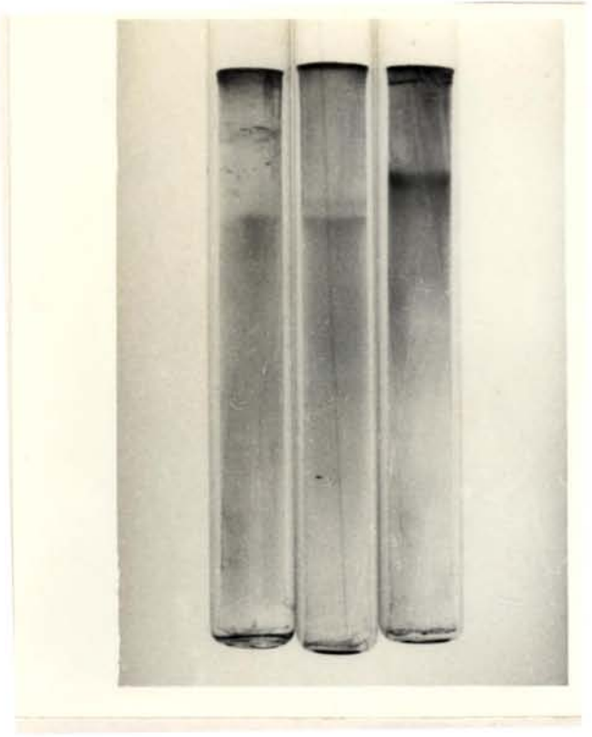

Zymogram patterns of Acid Phosphatase in M. cephalus
COCHIN
MADRAS
ORISSA
(-) LOC
0 mm
70 mm
(+)
I
II
S
F
F1
S1

PLATE 22

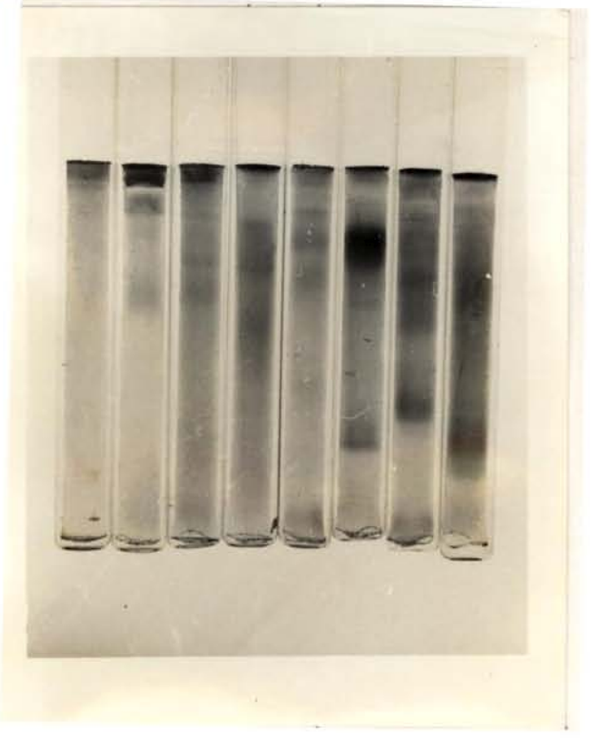
ACP

COCHIN



MADRAS

ORISSA



two polymorphic phenotypes in Cochin, a single fast band **F** (12-15 mm) and another single faster band **F1** (19-21 mm) whereas additional double band **SF** (5-7/12-15 mm) and **FF1** (12-15/19-21 mm) were also present in Madras. Five polymorphic phenotypes, namely, double banded **SF1** (5-7/19-21 mm) **S1F1** (8-10/19-21 mm) and **FF1** (12-15/19-21 mm); single banded **F** (12-15 mm) and **F1** (19-21 mm) were shown in Orissa. Thus these phenotypes were considered as genotypes produced by the four codominant alleles **S, S1, F** and **F1** in different combinations (TBL. 21). Madras and Orissa appeared to have their own rare alleles compared to Cochin, namely **S** alleles in Madras and **S** and **S1** alleles in Orissa (Fig. 68). The frequencies of these alleles in each region are shown in Table 21. The frequency patterns of the major alleles **F** and **F1** suggest striking difference existing among three populations of M. cephalus. When allelic frequencies of **F** and **F1** are almost equal ^{F} = 0.42, **F1** = 0.58) at Cochin, **F** allele was dominant, ^{F} = 0.62) at Madras whereas **F1** allele was dominant (0.64) at Orissa. The level of frequencies of **S** and **S1** ranging 0.06-0.13 suggest that they are rare alleles. These rare alleles were absent at Cochin. Six out of ten possible genotypes were observed. ACP genotype frequencies estimated for three places were also considerably deviated from that of observed values (TBL. 23). The two banded heterozygote suggests that the enzyme may be monomeric in nature.

4.5.2 ALDEHYDE OXIDASE (ADO)

The zymogram patterns of Aldehyde Oxidase (ADO) in M. cephalus tested from Cochin, Madras and Orissa are shown in the Figure 69 and Plate 23A. The zymogram showed a single zone of enzyme activity consisting of either a single or double banded phenotype in the populations tested from Cochin, Madras and Orissa.

Three polymorphic phenotypes: (1) a single slow band **S** (12-13 mm), (2) a double band **SF** (12-13/14-15 mm) and (3) a single fast band **F** (14-15 mm) were observed in Cochin, Madras and Orissa. A close comparison of these three phenotype variants suggested that they may be codominant di-allelic products at a single locus. The presumed genotypes are slow and fast single

FIGURE 69 AND PLATE 23A

Zymogram patterns of Aldehyde Oxidase in M. cephalus populations from Cochin, Madras and Orissa showed the common genotypes.

Locus	Alleles
I	S = 12-13 mm
	F = 14-15 mm

FIGURE 70 AND PLATE 23B

Zymogram patterns of Aldolase in M. cephalus populations from Cochin, Madras and Orissa showed the common genotypes.

Locus	Alleles
I	S = 0-3 mm
	F = 8-10, 8-11 mm

FIG. 69

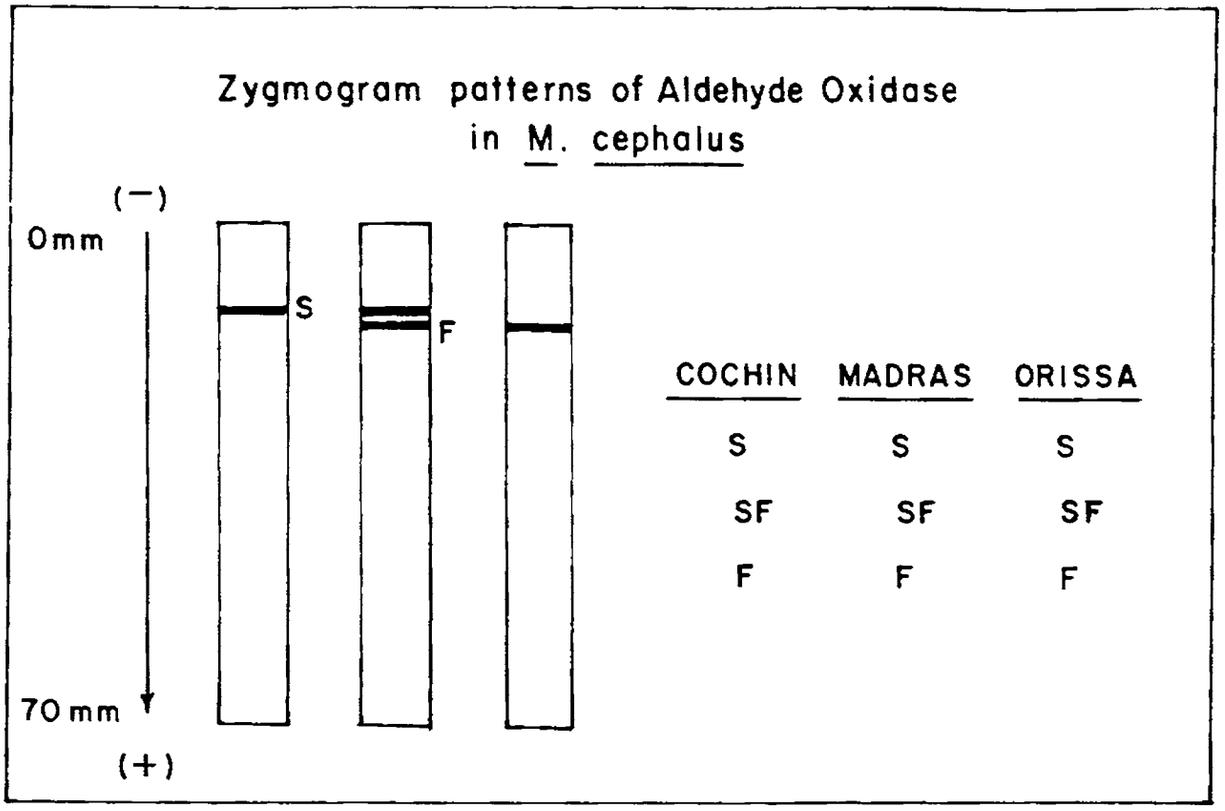

Zygmogram patterns of Aldehyde Oxidase
in M. cephalus
(−)
0mm
S
F
70 mm
(+)
COCHIN
MADRAS
ORISSA
S
S
S
SF
SF
SF
F
F
F

FIG. 70

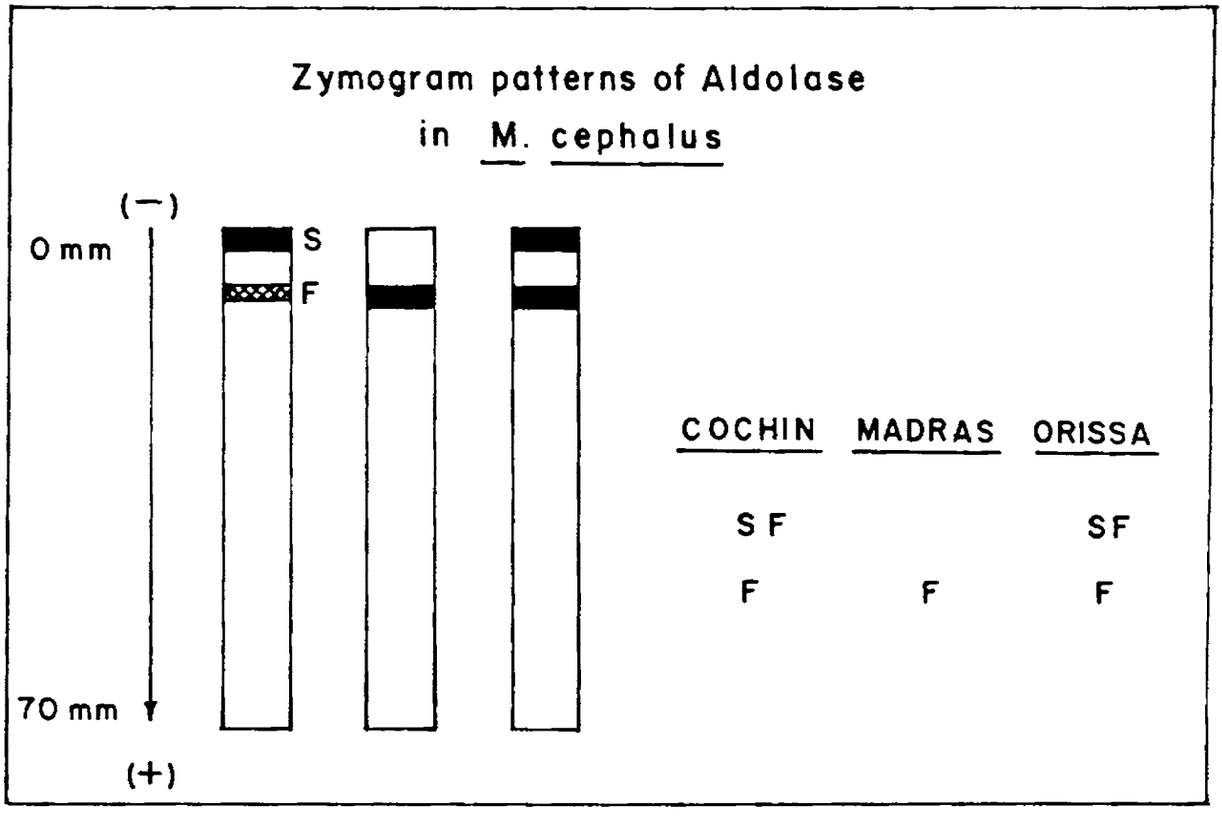
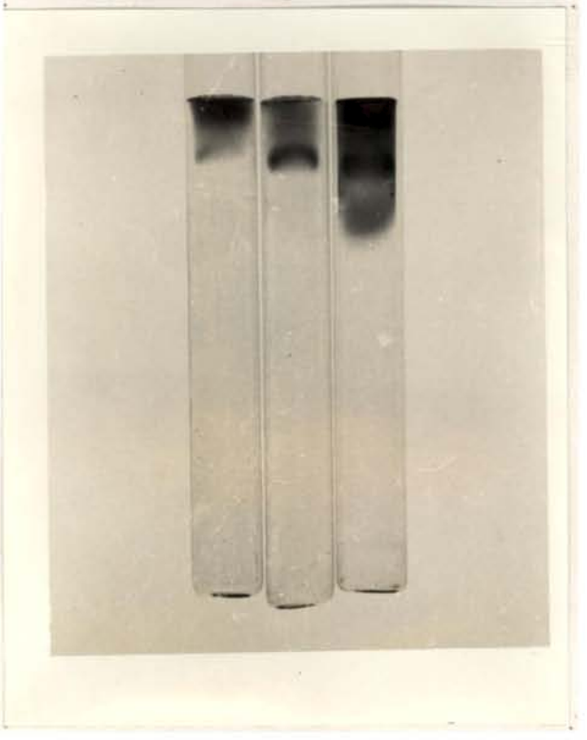

Zymogram patterns of Aldolase
in M. cephalus
(−)
0 mm
S
F
70 mm
(+)
COCHIN
MADRAS
ORISSA
S F
SF
F
F
F

PLATE 23

(A) ADO

(B) ALD



banded homozygotes and slow-fast double banded heterozygote. The two band heterozygote indicates monomeric enzyme structure of ADO in M. cephalus. The allelic frequencies calculated showed similar values for Cochin and Madras but different for Orissa these being 0.63 in Cochin and Madras while 0.50 in Orissa for the major **S** allele (TBL. 21). The values for genotype distribution showed excess homozygotes in Cochin and Orissa regions (TBL. 23).

4.5.3 ALDOLASE (ALD)

The zymogram patterns of Aldolase (ALD) in M. cephalus populations from Cochin, Madras and Orissa are shown in the Figure 70 and Plate 23B. The Cochin population showed two polymorphic phenotypes namely, double band **SF** (0-3/8-10 mm) and single band **F** (8-11 mm). The Madras population showed only single monomorphic **F** phenotype (8-11 mm) which was more intensely stained than that of Cochin (8-10 mm). On the other hand, Orissa population also exhibited two polymorphic phenotypes, **SF** (0-3/8-10, 8-11 mm) and **F** (8-11 mm) respectively. The two banded heterozygote indicates monomeric nature of the ALD enzyme. The allele frequencies and expected genotype frequencies for ALD calculated from the observed phenotypes are shown in Table 21 and 23. The allelic frequencies of ALD were significantly different in Cochin, Madras and Orissa (TBL. 21). The significance is reflected in frequency of the major allele F, it being 0.54 in Cochin, 1.00 in Madras and 0.76 in Orissa. The expected genotype values deviated significantly from that of observed values in Cochin ($P > 0.001$) and Orissa ($P < 0.01$) regions due to excess of heterozygotes.

4.5.4 ESTERASE (EST)

The zymogram patterns of esterase (EST) enzyme in M. cephalus populations showed three polymorphic phenotypes as shown in the Figure 71 and Plate 24A. These three phenotypes were composed of two banded **SS** (7-10/11-14 mm), three banded **SF** (7-9/11-14/16-20 mm) and two banded **FF** (11-14/16-20 mm) Cochin population showed only **SS** and **SF** phenotypes whereas Orissa showed all the three **SS**, **SF** and **FF** phenotypes. However, Madras

FIGURE 71 AND PLATE 24A

Zymogram patterns of Esterase in M. cephalus populations from Cochin, Madras and Orissa showed the common genotypes.

Locus	Alleles		
I	SS	=	7-10/11-14 mm
	SF	=	7-9/11-14/16-20 mm
	FF	=	11-14/16-20 mm

FIGURE 72 AND PLATE 24B

Zymogram patterns of Alpha-Glycerophosphate Dehydrogenase in M. cephalus populations from Cochin, Madras and Orissa showed the common genotypes.

Locus	Alleles		
I	S	=	3-6 mm
	SF	=	3-5/10-15/20-22 mm
	F	=	20-25 mm

FIG. 71

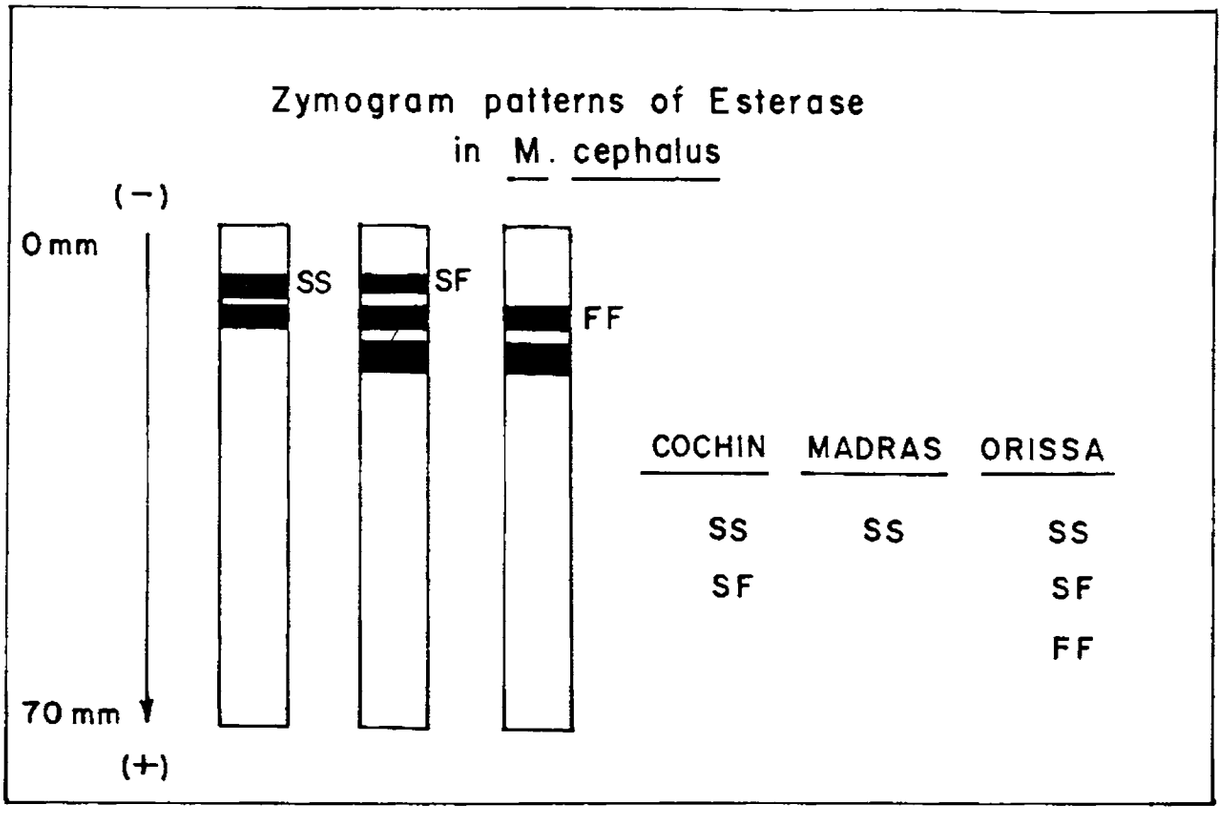

Zymogram patterns of Esterase
in M. cephalus
(−)
0 mm
70 mm
(+)
SS
SF
FF
COCHIN
MADRAS
ORISSA
SS
SF
SS
SS
SF
FF

FIG. 72

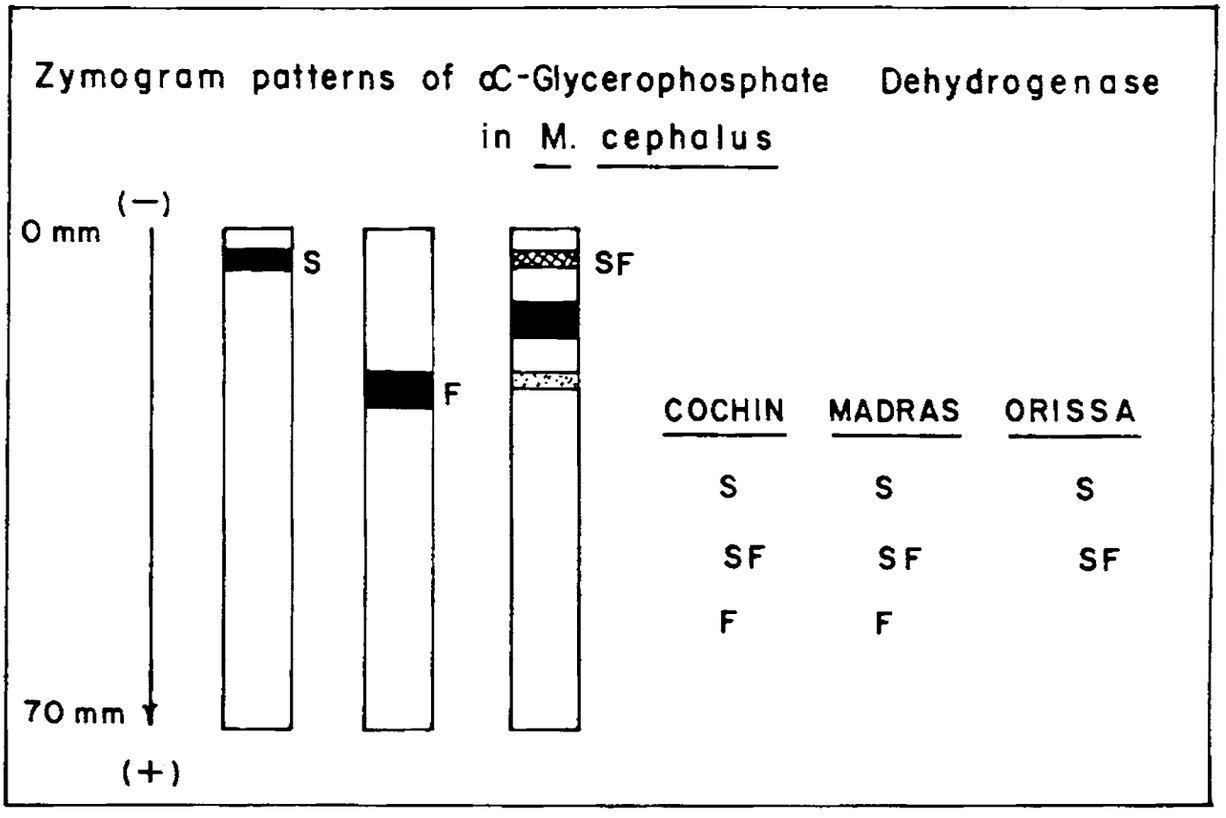
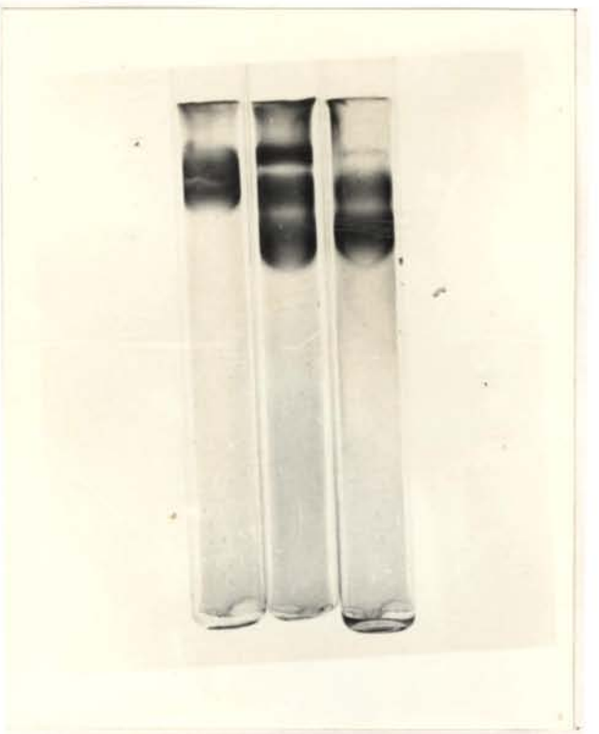

Zymogram patterns of αC-Glycerophosphate Dehydrogenase
in M. cephalus
(−)
0 mm
70 mm
(+)
S
F
SF
COCHIN
MADRAS
ORISSA
S
SF
F
S
SF
F
S
SF

PLATE 24

(A) EST



(B) GPD

showed only the **SS** phenotype. The two banded **SS** and **FF** phenotypes were scored as slow and fast homozygotes whereas three banded **SF** heterozygote was taken as heterozygote produced by these two banded homozygotes. The three banded heterozygote indicated that EST enzyme in the heart tissue of M. cephalus behaves as if a dimeric enzyme. The estimated allelic frequencies were significantly different in Cochin, Madras and Orissa (TBL. 21). Though major esterase allele in Cochin and Madras was **S**, it was **F** in Orissa. Frequencies of **S** allele were again considerably different between Cochin (0.83) and Madras (1.00) whereas it was only 0.13 in Orissa. It is interesting to note the strikingly different nature of allelic distribution in Cochin and Orissa, frequencies of **S** allele being 0.83 in Cochin and only 0.13 in Orissa, followed by frequencies of **F** allele being only 0.17 in Cochin and 0.87 in Orissa (TBL. 21). However, the expected genotype distribution was found in good agreement with H.W. equilibrium conditions showing a balanced polymorphism at EST locus (TBL. 23).

4.5.5 ALPHA-GLYCEROPHOSPHATE DEHYDROGENASE (GPD)

The zymogram patterns of Alpha-Glycerophosphate Dehydrogenase (GPD) in sample populations of M. cephalus tested from Cochin, Madras and Orissa are shown in the Figure 72 and Plate 24 B. The zymogram showed three polymorphic phenotypes in M. cephalus. These three phenotypes were composed of a single banded **S** (3-6 mm) tripple banded **SF** (3-5/10-15/20-22 mm) and single banded **F** (20-25 mm). These phenotype were scored as slow homozygote, slow-fast heterozygote and fast homozygote genotypes respectively. The three banded heterozygote structure indicates dimeric structure of the enzyme. Though all the three phenotypes were present in Cochin and Madras, the **F** phenotype was absent in Orissa. The calculated allelic frequencies were nearly similar between Cochin and Madras but considerably different in Orissa (TBL. 21). The frequencies of **F** allele being 0.50, 0.56 and 0.38 in Cochin, Madras and Orissa respectively. The genotype frequency distribution in Cochin and Orissa deviated considerably from that of expected ($P \geq 0.001$) due to excess of homozygotes in Cochin whereas due to excess of heterozygotes in Orissa (TBL. 23).

4.5.6 OCTANOL DEHYDROGENASE (ODH)

The zymogram patterns of Octanol Dehydrogenase (ODH) enzyme in M. cephalus are shown in the Figure 73 and Plate 26. Three polymorphic phenotypes composed of single banded **S, F** and three banded **SF** were detected, having band positions (2-7 mm), (11-14 mm) and (2-4/5-9/10-14 mm) respectively. In some of the specimens a four banded pattern was also noticed (PLT. 26). The **S** phenotype also appeared to show an additional minor fraction. The three banded phenotype was considered as heterozygote and single banded **S** and **F** as homozygotes according to their slow and fast band positions. The three banded heterozygote indicated that the enzyme is dimeric in structure. Cochin and Madras populations showed only three banded genotypes but not the homozygotes. Orissa showed all the three genotypes.

Allelic frequencies calculated from the observed genotypes are shown in the Table 21. As the phenotypes at Cochin and Madras were heterozygotes the frequencies of both alleles were equal. However, Orissa with all the three phenotypes showed different frequencies. The expected frequencies of genotypes deviated significantly in Cochin and Madras ($P > 0.001$) due to excess of heterozygotes (TBL. 23).

4.5.7 PYRROLIN DEHYDROGENASE (PDH)

The zymogram patterns of Pyrrolin Dehydrogenase (PDH) in sample populations of M. cephalus tested from Cochin, Madras and Orissa are shown in the Figure 76 and Plate 29. The zymogram showed a single zone of enzyme activity expressed by a single band in all populations. However, the phenotype variations in the form of single and double dose of enzyme activity indicated polymorphism at the enzyme locus. Thus, a narrow banded **S** phenotype (9-10 or 9-11 mm) and a broad banded **SF** phenotype (9-13 mm) were observed. The narrow banded **S** phenotype was scored as slow homozygote and the broad **SF** phenotype as heterozygote. The Cochin and Orissa populations showed both **S** and **SF** phenotypes whereas Madras population showed only **SF** phenotypes (FIG. 76). The allelic frequencies were almost similar between Cochin and

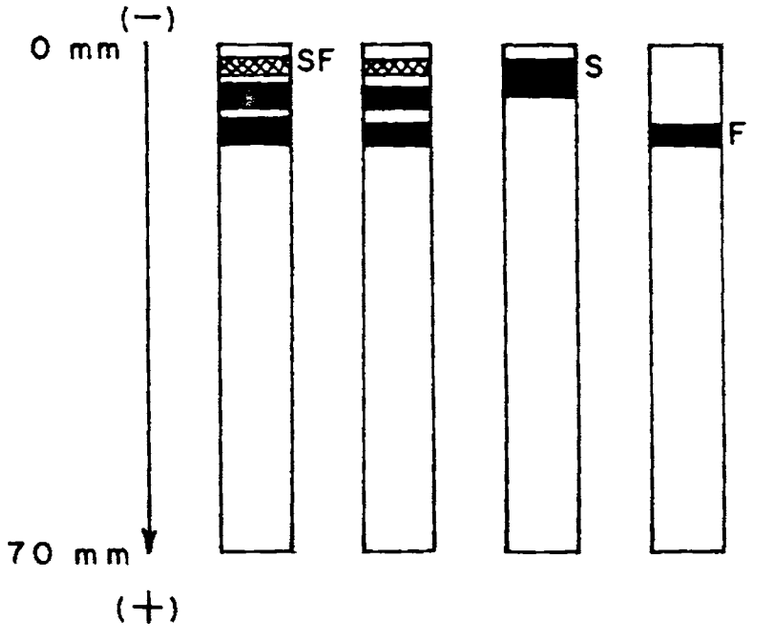
FIGURE 73 AND PLATE 26

Zymogram patterns of Octanol Dehydrogenase in M. cephalus populations from Cochin, Madras and Orissa showed the common genotypes.

Locus	Alleles		
I	S	=	2-7 mm
	SF	=	2-4/5-9/10-14 mm
	F	=	11-14 mm

FIG. 73

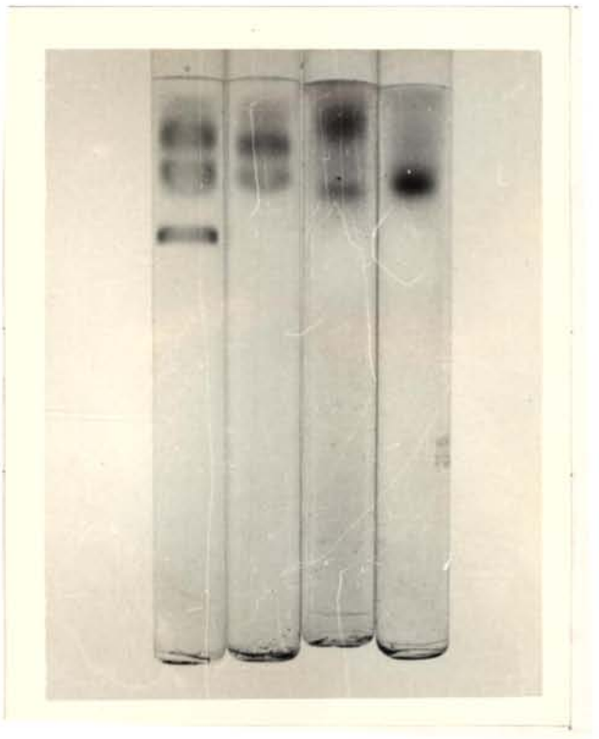
Zymogram patterns of Octanol Dehydrogenase in M. cephalus



COCHIN	MADRAS	ORISSA
		S
SF	SF	SF
		F

PLATE 26

ODH



Orissa but considerably different at Madras (TBL. 21). As all specimens tested from Madras were heterozygotes the frequencies of both alleles were found to be equal. The nature of genotype distributions showed considerable deviation from the expected ($P > 0.001$) due to excess of heterozygote only at Madras whereas other regions were showing a balanced polymorphism at the enzyme locus (TBL. 23). The enzyme appears to be monomeric in structure.

4.5.8 TETRAZOLIUM OXIDASE (TZO)

During detection of specific enzymes like ADH, ADO, ALD, ODH etc. certain gel areas were also shown as having bleached areas of phenotype nature. These phenotype variations were reported here as Tetrazolium Oxidase enzyme. TZO enzyme activity in Orissa sample could not be resolved well due to some unknown reason. The phenotypes observed in Cochin and Madras consisted of a single dose **S** (7-12 mm) and more than a double dose **SF** (8-18 mm) which always found migrating just faster than the enzyme for which the actual test was conducted (FIG. 77). The frequencies of the two alleles were similar ($S = 0.71$, $F = 0.29$) in both Cochin and Madras populations (TBL. 21). The expected frequencies of genotypes deviated significantly in Cochin and Madras ($P < 0.001$) due to excess of heterozygotes (TBL. 23).

4.5.9 EYE LENS LACTATE DEHYDROGENASE (LDH (E))

Plate 25A shows the patterns of eye lens lactate dehydrogenase enzyme in M. cephalus. All the specimens tested from Cochin, Madras and Orissa showed identical five banded patterns suggesting its non-polymorphic nature in the species. LDH (E) showed only one locus.

4.5.10 MUSCLE LACTATE DEHYDROGENASE (LDH (M))

Plate 25B shows the patterns of muscle lactate dehydrogenase in M. cephalus. Muscle showed two LDH bands in all the specimens tested from Cochin, Madras and Orissa. These two bands were presumed to be the products of two separate locus. Muscle LDH also showed no polymorphic forms in the species.

FIGURE 76 AND PLATE 29

Zymogram patterns of Pyrroline Dehydrogenase in M. cephalus populations from Cochin, Madras and Orissa showed the common genotypes.

Locus	Alleles
I	S = 9-10, 9-11 mm
	SF = 9-13 mm

FIGURE 77

Zymogram patterns of Tetrazolium Oxidase in M. cephalus populations from Cochin, Madras and Orissa showed the common genotypes.

Locus	Alleles
I	S = 7-12 mm
	SF = 8-18 mm

FIG. 76

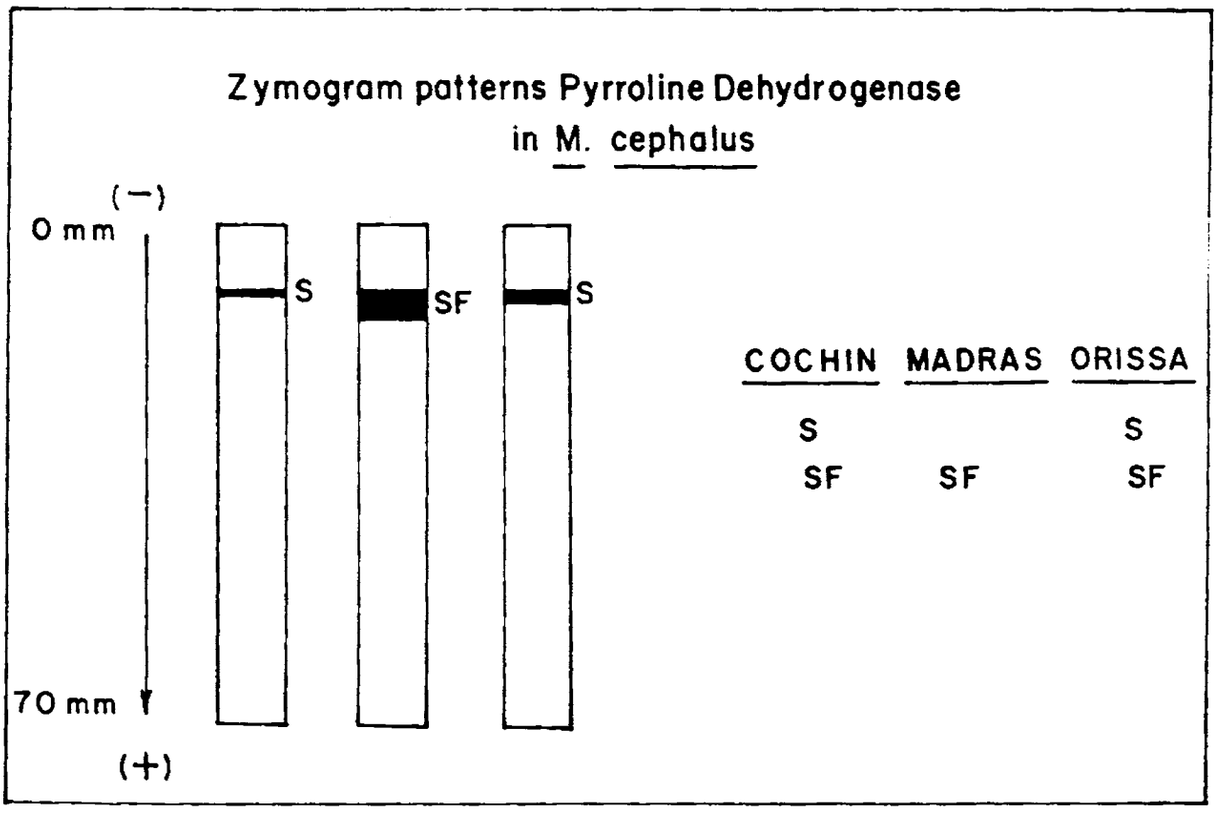

Zymogram patterns Pyrroline Dehydrogenase
in M. cephalus
(−)
0 mm
S
SF
S
70 mm
(+)
COCHIN
MADRAS
ORISSA
S
S
SF
SF
SF

FIG. 77

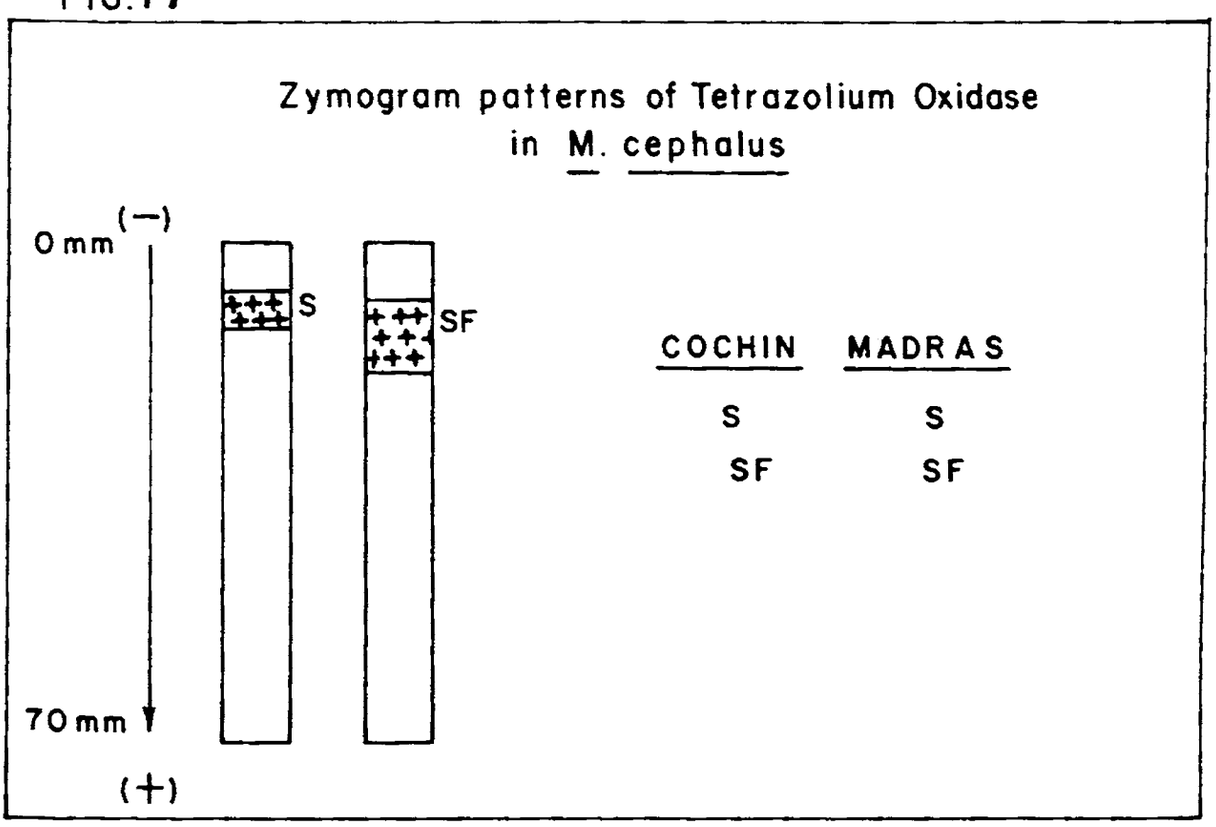

Zymogram patterns of Tetrazolium Oxidase
in M. cephalus
(−)
0 mm
S
SF
70 mm
(+)
COCHIN
MADRAS
S
S
SF
SF

PLATE 29

PDH

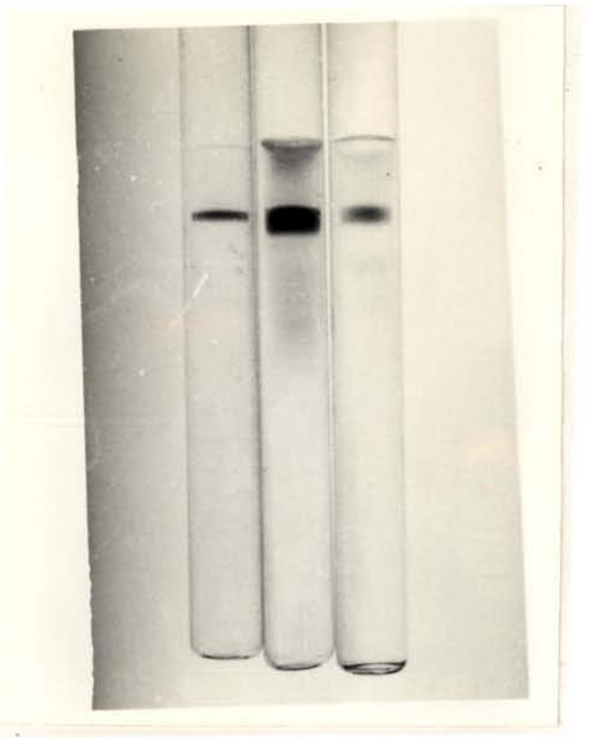


PLATE 25A

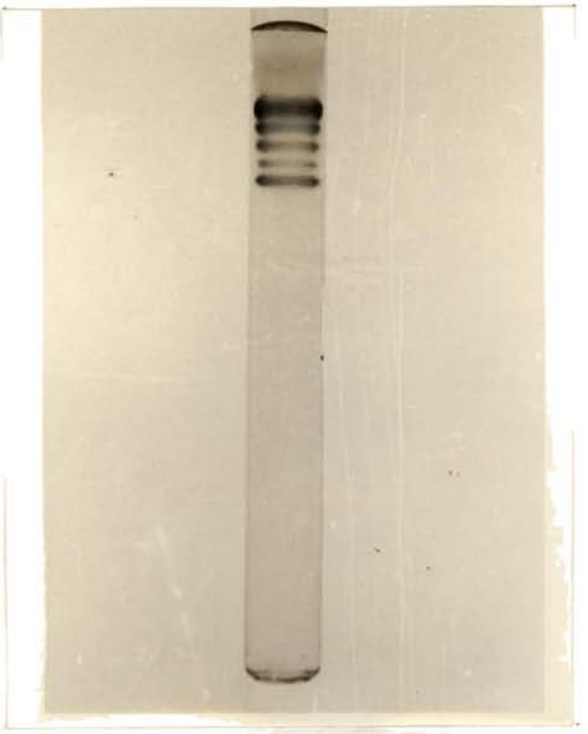
Eye lens Lactate Dehydrogenase patterns in M. cephalus populations from Cochin, Madras and Orissa showed the monomorphic genotype.

PLATE 25B

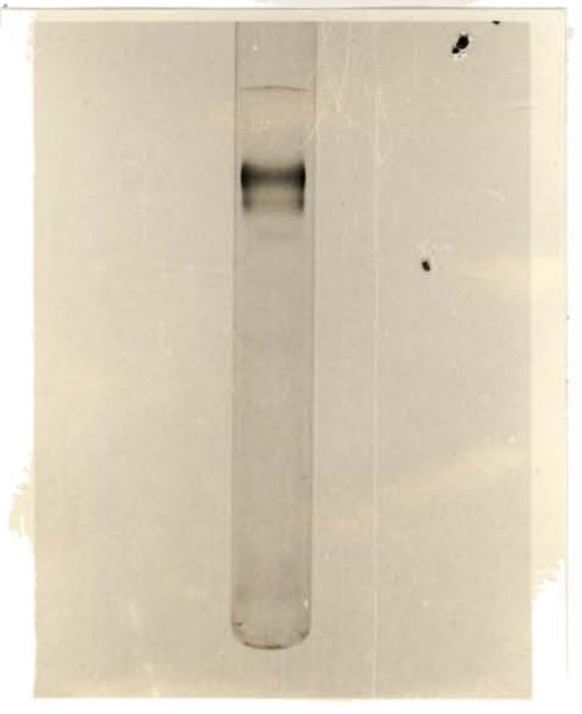
Muscle Lactate Dehydrogenase patterns in M. cephalus populations from Cochin, Madras and Orissa showed the monomorphic genotype.

PLATE 25

(A) EYE LENS-LDH



(B) MUSCLE-LDH



4.6 PROTEINS/ENZYMES TESTED ONLY FROM COCHIN

During the preliminary investigation of electrophoretic patterns of different protein systems in M. cephalus the following could be tested only from Cochin area.

Serum samples tested from a few specimens from Cochin showed highly variable inconsistent patterns as shown in Plate 31A. Hence it was not tested from other areas. The observed variability in the serum protein pattern may indicate non-genetic polymorphism.

Similarly, the enzymes like ADH, MDH and SDH could be tested only from Cochin area. All these three enzymes appeared to show certain phenotype variations in Cochin. Sorbitol dehydrogenase variations could not be scored as genetic variations (PLT. 31B). However, phenotype variations found in ADH and MDH enzymes appeared to be of genetic nature (PLT. 30A, B). However, these enzymes could not be tested from other regions.

4.7 ANALYSIS OF DATA

4.7.1 AVERAGE POLYMORPHISM

Proportion of polymorphic loci observed in different populations of M. cephalus and average polymorphism in each population and average in M. cephalus were calculated and presented in Table 20. A total of twenty one loci in Cochin and Madras and twenty loci in Orissa were considered for calculation. Average polymorphism in each population and, hence, in M. cephalus was found to be very high. It was 0.72, 0.61 and 0.69 in Cochin, Madras and Orissa respectively. Thus the average is 0.67 (TBL. 20) for M. cephalus species.

4.7.2 AVERAGE NUMBER OF ALLELES

A total of twenty one loci consisting of seventeen polymorphic and four monomorphic loci (20 only for Orissa) were tested in M. cephalus (TBL. 20, 21). The number of alleles observed in each population at each locus and

PLATE 30A

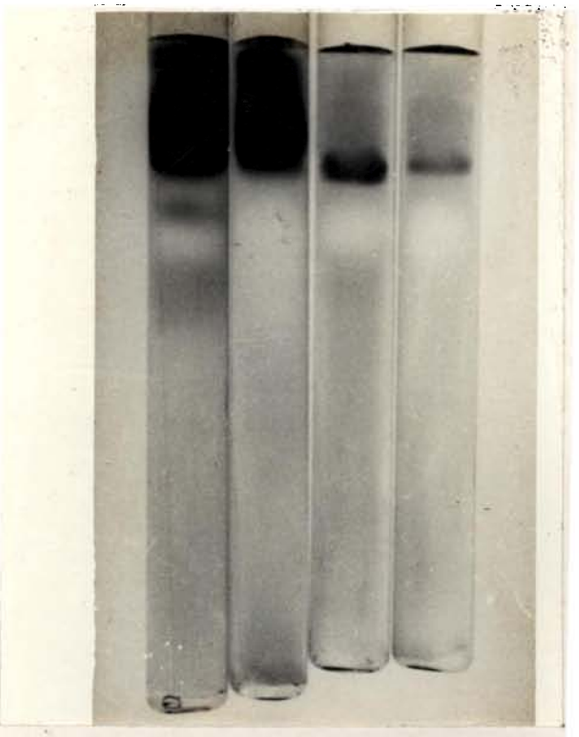
Alcohol Dehydrogenase patterns in M. cephalus population from Cochin.

PLATE 30B

Malate Dehydrogenase patterns in M. cephalus population from Cochin.

PLATE 30

(A) ADH - COCHIN



(B) MDH- COCHIN

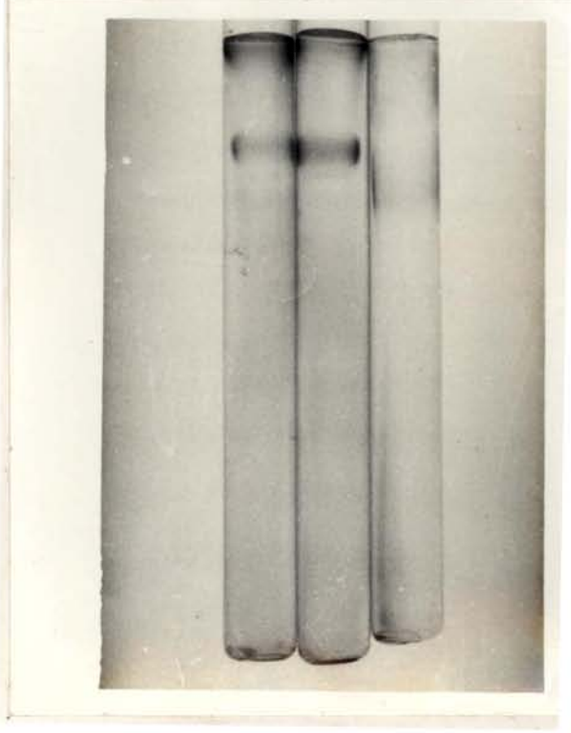


PLATE 31A

Serum Protein patterns in M. cephalus population from Cochin.

PLATE 31B

Sorbital Dehydrogenase patterns in M. cephalus population from Cochin.

PLATE 31

(A) SERUM-PRO — COCHIN

(B) SDH - COCHIN

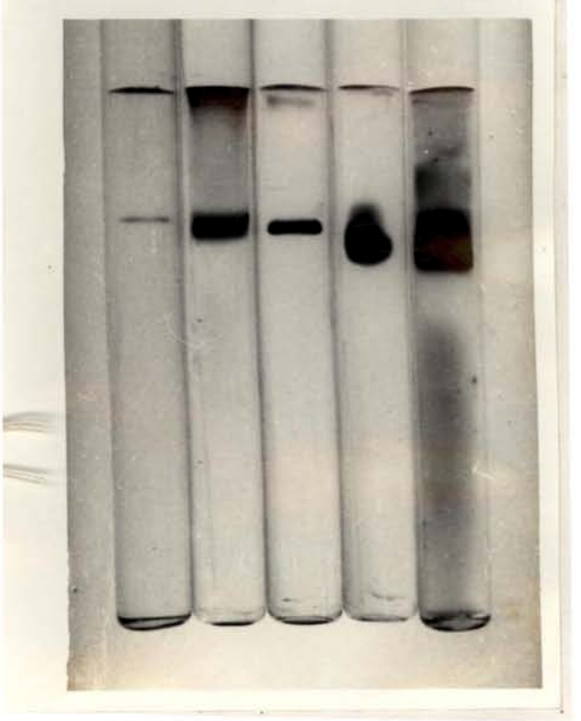


TABLE 20*

Comparison of Average Polymorphic Loci in Mugil cephalus L. Populations.

Sl. No.	Loci	No. of Loci present			No. of Polymorphic Loci			Average No. of Polymorphic Locus		
		COH	MAD	ORI	COH	MAD	ORI	COH	MAD	ORI
1.	ACP	2	2	2	1	1	1	0.50	0.50	0.50
2.	ADO	1	1	1	1	1	1	1.00	1.00	1.00
3.	ALD	1	1	1	1	0	1	1.00	0.00	1.00
4.	EST	1	1	1	1	0	1	1.00	0.00	1.00
5.	GPD	1	1	1	1	1	1	1.00	1.00	1.00
6.	LDH (E)	1	1	1	0	0	0	0.00	0.00	0.00
7.	LDH (M)	2	2	2	0	0	0	0.00	0.00	0.00
8.	ODH	1	1	1	1	1	1	1.00	1.00	1.00
9.	PRO (E)	5	5	5	3	4	3	0.60	0.80	0.60
10.	PRO (M)	4	4	4	2	4	2	0.50	1.00	0.50
11.	PDH	1	1	1	1	1	1	1.00	1.00	1.00
12.	TZO	1	1	-	1	1	-	1.00	1.00	-
	Total	21	21	20	13	14	12	8.60/12	7.30/12	7.60/11
	Average No. of Polymorphic Loci in each Population						=	0.72	0.61	0.69
	Average No. of Polymorphic Loci in Mugil cephalus						=	0.67		

* Refer Page xvi for explanation of abbreviations

TABLE 21*

Allele Frequencies at 21 Loci (20 Loci for Orissa) in three Populations of Mugil cephalus L.

Sl.No.	Locus	Allele	Populations		
			COH	MAD	ORI
1.	ACP I	S	1.00	1.00	1.00
2.	ACP II	S	0.00	0.10	0.06
		S1	0.00	0.00	0.13
		F	0.42	0.62	0.17
		F1	0.58	0.28	0.64
3.	ADO	S	0.63	0.63	0.50
		F	0.37	0.37	0.50
4.	ALD	S	0.46	0.00	0.24
		F	0.54	1.00	0.76
5.	EST	S	0.83	1.00	0.13
		F	0.17	0.00	0.87
6.	GPD	S	0.50	0.44	0.62
		F	0.50	0.56	0.38
7.	LDH (E)	?	1.00	1.00	1.00
8.	LDH (M) I	?	1.00	1.00	1.00
9.	LDH (M) II	?	1.00	1.00	1.00
10.	ODH	S	0.50	0.50	0.43
		F	0.50	0.50	0.57
11.	PRO (E) I	S	1.00	0.90	1.00
		F	0.00	0.10	0.00
12.	PRO (E) II	S	1.00	0.90	0.87
		F	0.00	0.10	0.13
13.	PRO (E) III	S	0.50	0.50	0.38
		F	0.50	0.50	0.62
14.	PRO (E) IV	S	0.25	0.00	0.00
		F	0.75	1.00	1.00
15.	PRO (E) V	S	0.62	0.48	0.38
		F	0.38	0.52	0.62
16.	PRO (M) I	S	0.00	0.63	0.00
		F	1.00	0.37	1.00
17.	PRO (M) II	S	0.54	0.58	0.50
		F	0.46	0.42	0.50
18.	PRO (M) III	S	0.50	0.70	0.50
		F	0.50	0.30	0.50
19.	PRO (M) IV	S	1.00	0.80	0.00
		F	0.00	0.20	1.00
20.	PDH	S	0.83	0.50	0.88
		F	0.17	0.50	0.12
21.	TZO	S	0.71	0.71	-
		F	0.29	0.29	-

* Refer Page xvi for explanation of abbreviations

TABLE 22*

Comparison of Average Number of Alleles at 21 Loci (20 for Orissa) in Mugil cephalus L. Populations.

Sl. No.	Loci	Total Loci	Alleles Cochin				Alleles Madras				Alleles Orissa					No. of Average Alleles		
			S	F	F1	Total Alleles	S	F	F1	Total Alleles	S	S1	F	F1	Total Alleles	COH	MAD	ORI
1.	ACP	2	1	1	1	3	2	1	1	4	2	1	1	1	5	1.50	2.00	2.50
2.	ADO	1	1	1		2	1	1		2	1		1		2	2.00	2.00	2.00
3.	ALD	1	1	1		2	1	1		2	1		1		2	2.00	2.00	2.00
4.	EST	1	1	1		2	1	1		2	1		1		2	2.00	2.00	2.00
5.	GPD	1	1	1		2	1	1		2	1		1		2	2.00	2.00	2.00
6.	LDH(E)	1				1				1					1	1.00	1.00	1.00
7.	LDH(M)	2				2				2					2	1.00	1.00	1.00
8.	ODH	1	1	1		2	1	1		2	1		1		2	2.00	2.00	2.00
9.	PRO(E)	5	5	3		8	4	5		9	4		4		8	1.60	1.80	1.60
10.	PRO(M)	4	3	3		6	4	4		8	3		3		6	1.50	2.00	1.50
11.	PDH	1	1	1		2	1	1		2	1		1		2	2.00	2.00	2.00
12.	TZO	1	1	1		2	1	1		2	-		-		-	2.00	2.00	--
	Total	21														20.60	21.80	19.60
	Average number of Alleles in each population															1.72	1.82	1.78
	Average number of Alleles in Mugil cephalus															1.77		

* Refer Page xvi for explanation of abbreviations

TABLE 23*

Comparison of Observed and Expected (Hardy-Wienberg Law) Genotype Frequencies in Mugil cephalus Populations.

Expected Values are given in Parenthesis.

Sl. No.	Locus	Genotype	COCHIN			MADRAS			ORISSA		
			OBS	(Exp)	Chi-square	OBS	(Exp)	Chi-square	OBS	(Exp)	Chi-square
1.	ACP II	SS	-	-	-	0	(0.52)	0.52	0	(0.17)	0.17
		S1S1	-	-	-	-	-	-	0	(0.81)	0.81
		FF	20	(8.47)	15.70	22	(19.99)	0.20	2	(1.39)	0.27
		F1F1	28	(16.14)	8.71	10	(4.08	8.59	16	(19.66)	0.68
		SS1	-	-	-	-	-	-	0	(0.75)	0.75
		SF	-	-	-	10	(6.45)	1.95	0	(0.98)	0.98
		SF1	-	-	-	0	(2.91)	2.91	6	(3.69)	1.45
		S1F	-	-	-	-	-	-	0	(2.12)	2.12
		S1F1	-	-	-	-	-	-	12	(7.99)	2.01
		FF1	0	(23.39)	23.39	10	(18.05)	3.59	12	(10.44)	0.23
		TOL			47.80			17.76			9.47
				P > 0.001 df 6			P < 0.01 df 7			P < 0.05 df 6	
2.	ADO	SS	24	(19.05)	1.29	16	(19.05)	0.49	18	(12.00)	3.00
		SF	12	(22.38)	4.81	28	(22.38)	1.41	12	(24.00)	6.00
		FF	12	(6.57)	4.49	4	(6.57)	1.01	18	(12.00)	3.00
		TOL			10.59			2.91			12.00
				P < 0.001 df 1						P > 0.001 df 1	
3.	ALD	SS	0	(10.16)	10.16	-	-	-	0	(3.28)	3.28
		SF	44	(23.84)	17.05	-	-	-	27	(20.79)	1.85
		FF	4	(13.99)	7.13	48	(48)	0	30	(32.93)	0.26
		TOL			34.34						5.39
				P > 0.001 df 1						P < 0.01	
4.	EST	SS	32	(33.06)	0.03	48	(48)	0	2	(0.81)	1.75
		SF	16	(13.54)	0.45	-	-	-	8	(10.86)	0.75
		FF	0	(1.38)	1.38	-	-	-	38	(36.33)	0.08
		TOL			1.86						2.58
5.	GPD	SS	20	(12.00)	5.33	12	(9.29)	0.79	12	(18.45)	2.25
		SF	8	(24.00)	10.67	18	(23.66)	1.35	36	(22.62)	7.91
		FF	20	(12.00)	5.33	18	(15.06)	0.57	0	(6.93)	6.93
		TOL			21.33			2.71			17.09
				P > 0.001 df 1						P > 0.001 df 1	

* Refer Page xvi for explanation of abbreviations

TABLE 23* Continued.

Sl. No.	Locus	Genotype	COCHIN			MADRAS			ORISSA		
			OBS	(Exp)	Chi-square	OBS	(Exp)	Chi-square	OBS	(Exp)	Chi-square
6.	ODH	SS	0	(12.00)	12.00	0	(12.00)	12.00	8	(8.14)	0.00
		SF	48	(24.00)	24.00	48	(24.00)	24.00	22	(21.56)	0.01
		FF	0	(12.00)	12.00	0	(12.00)	12.00	14	(14.30)	0.01
		TOL			48.00			48.00			0.02
				P > 0.001 df 1			P > 0.001 df 1				
7.	PRO(E) I	SS	48	(48)	0	40	(38.88)	0.03	48	(48)	0
		SF	-	-	-	6	(8.64)	0.81	-	-	-
		FF	-	-	-	2	(0.48)	4.81	-	-	-
		TOL						5.65			
							P < 0.01 df 1				
8.	PRO(E) II	SS	48	(48)	0	40	(38.88)	0.03	36	(36.33)	0.00
		SF	-	-	-	6	(8.64)	0.81	12	(10.86)	0.12
		FF	-	-	-	2	(0.48)	4.81	0	(0.81)	0.81
		TOL						5.65			0.93
							P < 0.01 df 1				
9.	PRO(E) III	SS	0	(12.00)	12.00	0	(12.00)	12.00	0	(6.93)	6.93
		SF	48	(24.00)	24.00	48	(24.00)	24.00	36	(22.62)	7.91
		FF	0	(12.00)	12.00	0	(12.00)	12.00	12	(18.45)	2.25
		TOL			48.00			48.00			17.09
				P > 0.001 df 1			P > 0.001 df 1			P > 0.001 df 1	
10.	PRO(E) IV	SS	0	(3.00)	3.00	-	-	-	-	-	-
		SF	24	(18.00)	2.00	-	-	-	-	-	-
		FF	24	(27.00)	0.33	48	(48)	0	48	(48)	0
		TOL			5.33						
				P < 0.01 df 1							
11.	PRO(E) V	SS	12	(18.45)	2.25	0	(11.06)	11.06	0	(6.93)	6.93
		SF	36	(22.62)	7.91	46	(23.96)	20.27	36	(22.62)	7.91
		FF	0	(6.93)	6.93	2	(12.98)	9.29	12	(18.45)	2.25
		TOL			17.09			40.62			17.09
				P > 0.001 df 1			P > 0.001 df 1			P > 0.001 df 1	

* Refer Page xvi for explanation of abbreviations

TABLE 23* Continued

Sl. No.	Locus	Genotype	COCHIN			MADRAS			ORISSA		
			OBS	(Exp)	Chi - square	OBS	(Exp)	Chi- square	OBS	(Exp)	Chi- square
12.	PRO(M) I	SS	-	-	-	34	(33.34)	0.01	-	-	-
		SF	-	-	-	37	(39.16)	0.12	-	-	-
		FF	48	(48)	0	13	(11.50)	0.20	48	(48)	0
								0.33			
13.	PRO(M) II	SS	4	(13.99)	7.13	13	(28.26)	8.24	0	(12.00)	12.00
		SF	44	(23.84)	17.05	71	(40.92)	22.11	48	(24.00)	24.00
		FF	0	(10.16)	10.16	0	(14.82)	14.82	0	(12.00)	12.00
		TOL			34.34			45.17			48.00
				P > 0.001 df 1			P > 0.001 df 1			P > 0.001 df 1	
14.	PRO(M) III	SS	0	(12.00)	12.00	34	(41.16)	1.25	0	(12.00)	12.00
		SF	48	(24.00)	24.00	50	(35.28)	6.14	48	(24.00)	24.00
		FF	0	(12.00)	12.00	0	(7.56)	7.56	0	(12.00)	12.00
		TOL			48.00			14.95			48.00
				P > 0.001 df 1			P > 0.001 df 1			P > 0.001 df 1	
15.	PRO(M) IV	SS	48	(48)	0	0	(3.36)	3.36	-	-	-
		SF	-	-	-	34	(26.88)	1.89	-	-	-
		FF	-	-	-	50	(53.76)	0.26	48	(48)	0
		TOL						5.51			
								P < 0.01 df 1			
16.	PDH	SS	32	(33.06)	0.03	0	(12.00)	12.00	36	(37.17)	0.04
		SF	16	(13.55)	0.44	48	(24.00)	24.00	12	(10.14)	0.34
		FF	0	(1.39)	1.39	0	(12.00)	12.00	0	(0.69)	0.69
		TOL			1.89			48.00			1.07
								P > 0.001 df 1			
17.	TZO	SS	20	(24.20)	0.73	20	(24.20)	0.73	-	-	-
		SF	28	(19.77)	3.43	28	(19.77)	3.43	-	-	-
		FF	0	(4.03)	4.03	0	(4.03)	4.03	-	-	-
		TOL			8.19			8.19			
				P < 0.001 df 1			P < 0.001 df 1				

* Refer Page xvi for explanation of abbreviations

TABLE 24*

Heterozygosity at 21 loci (20 for Orissa) in three Populations of Mugil cephalus L.

Sl. No.	Number of Loci		Heterozygosity		
			Cochin	Madras	Orissa
1.	ACP I	1.	0.00	0.00	0.00
2.	ACP II	2.	0.00	0.38	0.63
3.	ADO	3.	0.25	0.58	0.25
4.	ALD	4.	0.92	0.00	0.47
5.	EST	5.	0.33	0.00	0.17
6.	GPD	6.	0.50	0.38	0.75
7.	LDH(E)	7.	0.00	0.00	0.00
8.	LDH(M)	8-9.	0.00	0.00	0.00
9.	ODH	10.	1.00	1.00	0.50
10.	PRO(E) (average for 5 loci)	11-15	0.45	0.44	0.35
11.	PRO(M) (average for 4 loci)	16-19	0.48	0.57	0.50
12.	PDH	20.	0.33	1.00	0.25
13.	TZO	21	0.58	0.58	--
		Total	4.84	4.93	3.87
	Average Heterozygosity in COH/MAD/ORI Population		0.37	0.38	0.32
	Heterozygosity in Mugil cephalus		0.36		

* Refer Page xvi for explanation of abbreviations

TABLE 25*

Excess of Homozygotes/Heterozygotes at significant level in Mugil cephalus Populations

Sl. No.	Locus	COCHIN		MADRAS		ORISSA	
		HOM	HET	HOM	HET	HOM	HET
1.	ACP II	+	-	+	-	-	-
2.	ADO	+	-	-	-	+	-
3.	ALD	-	+	-	-	-	-
4.	EST	-	-	-	-	-	-
5.	GPD	+	-	-	-	-	-
6.	ODH	-	+	-	+	-	-
7.	PRO(E) I	-	-	-	-	-	-
8.	PRO(E) II	-	-	-	-	-	-
9.	PRO(E) III	-	+	-	+	-	+
10.	PRO(E) IV	-	-	-	-	-	-
11.	PRO(E) V	-	+	-	+	-	+
12.	PRO(M) I	-	-	-	-	-	-
13.	PRO(M) II	-	+	-	+	-	+
14.	PRO(M) III	-	+	-	+	-	+
15.	PRO(M) IV	-	-	-	+	-	-
16.	PDH	-	-	-	+	-	-
17.	TZO	-	+	-	+	-	-
	Total	3	7	1	8	1	4

* Refer Page xvi for explanation of abbreviations

\+ = Excess of Homozygotes/Heterozygotes

P Values at significant level (Refer TBL. 23)

TABLE 26*

Paired comparison of values of Genetic Identity (I) and Distance (D) between Cochin, Madras and Orissa Populations of Mugil cephalus L.

Distance values are given in Parenthesis:

Sl. No.	Loci and Number		Cochin/Madras (I)	(D)	Cochin/Orissa (I)	(D)	Cochin/Orissa (I)	(D)
1.	PRO (E)	5 (average)	0.979	(0.0212)	0.795	(0.2294)	0.952	(0.0492)
2.	PRO (M)	4 (average)	0.556	(0.5870)	0.749	(0.2890)	0.848	(0.1649)
3.	ACP I	1	1.000	(0.0000)	1.000	(0.0000)	1.000	(0.0000)
4.	ACP II	1	0.868	(0.1416)	0.933	(0.0694)	0.633	(0.4573)
5.	ADO	1	1.000	(0.0000)	0.967	(0.0336)	0.967	(0.0336)
6.	ALD	1	0.762	(0.2718)	0.923	(0.0823)	0.954	(0.0471)
7.	EST	1	0.979	(0.0212)	0.348	(1.0556)	0.148	(1.9105)
8.	GPD	1	0.992	(0.0080)	0.973	(0.0274)	0.938	(0.0640)
9.	LDH (E)	1	1.000	(0.0000)	1.000	(0.0000)	1.000	(0.0000)
10.	LDH (M) I	1	1.000	(0.0000)	1.000	(0.0000)	1.000	(0.0000)
11.	LDH (M) II	1	1.000	(0.0000)	1.000	(0.0000)	1.000	(0.0000)
12.	ODH	1	1.000	(0.0000)	0.934	(0.0683)	0.934	(0.0683)
13.	PDH	1	0.835	(0.1803)	0.999	(0.0010)	0.796	(0.2282)
	Total	20	11.971	(1.2311)	11.619	(1.8560)	11.170	(3.0231)
	Population average		0.921	(0.0947)	0.894	(0.1427)	0.859	(0.2325)
	Species average		0.891	(0.1566)				

* Refer Pages xvi for explanation of abbreviations

their average are given in Table 22. Thus average in respect of all loci for Cochin, Madras and Orissa is 1.72, 1.82 and 1.78 respectively, whereas, average for M. cephalus is 1.77 (TBL. 22).

4.7.3 HETEROZYGOSITY

The proportions of heterozygotes at each locus and their average for all the twenty-one loci in each population and in M. cephalus species are given in Table 24. Average heterozygosity is considerably high in each population and, hence, in the species itself, these being 0.37, 0.36 and 0.30 in Cochin, Madras and Orissa respectively and 0.34 in M. cephalus species (TBL.24).

4.7.4 GENETIC IDENTITY (I) AND DISTANCE (D)

The method followed is that of Nei's (1975) as described by Utter (1987). Table 26 shows computed values of genetic identity and corresponding genetic distance for three different M. cephalus populations. These values were obtained after 54 paired comparisons involving 20 loci (39 paired comparisons and 13 loci only when multi loci like PRO (E) and PRO (M) were considered as one each because of their averages as shown in Table 26). These values indicate that the highest average genetic identity (I) is shared by Cochin and Madras populations, the value being 0.921 whereas the least identity existed between Madras and Orissa, it being 0.859. The identity value between Cochin and Orissa was a medium type. Similarly, the distance (D) values obtained for these regions also show comparable differences, these being 0.095 between Cochin and Madras, 0.143 between Cochin and Orissa, 0.233 between Madras and Orissa (TBL. 26). The average I and D values for the species are 0.891 and 0.157 respectively. Thus the highest genetic distance exists between Madras and Orissa.

5. DISCUSSION

A unit of fish stock concept is of fundamental importance in fisheries research and management. It has been a topic of detailed discussion, definition and modification of its definition since serious thoughts were given to it by fishery biologists and geneticists throughout the world (Marr, 1957; Muzinic and Marr, 1960). Because the concept of scientific exploitation of fish resources always based on some form of fishery units, like sub-species, races, varieties, populations or stocks etc. The importance of modern stock concept was further emphasized in ICES special meeting (de Ligny, 1971) and in the recent "Proceedings of the International Symposium" (Special issue: Can. J. Fish. Aquat. Sci., 38(12)) where continuing efforts were made to define and discuss unit stock concept more accurately. However, the importance of the concept of unit fish stock has not been changed to any extent, though, the techniques applied to demonstrate the existance of hitherto unknown natural fishery units vary widely. The techniques varied from the traditional morphometric, meristic, tagging, behavioural to modern serological, immunological, cytogenetic and biochemical genetic methods (Ihssen et al., 1981).

In view of the high economic importance of M. cephalus throughout the world, it has been a favourite species for investigations of various nature (Oren, 1981). Though M. cephalus is being heavily exploited throughout its range of distribution along the west and east coast of India, scientific informations on the biochemical genetic stock structure of the species are not available. Hence, the objective of present investigation was to study the biochemical genetics of M. cephalus with a view to understanding the unit stock structure of the species. The biochemical population genetic characteristics of the species in the form of general proteins and enzymes have been studied analysed and reported in the present investigation for the first time.

In biochemical genetic investigation, as in any other research programmes involving different parameters like equipments, chemicals of differeent nature, other physical parameters and even the experimental animals themselves, it is highly important to standardize all these parameters to the maximum possible extent to exclude artefacts or errors at any stage of the experiments.

Giving due considerations to the above standard practice, all the experiments conducted for collecting basic genetic data were standardized to the maximum possible extent, mainly with regard to correct species identification, electrophoretic resolution of different protein fractions and avoidance of ontogenetic variations. These procedures helped in correct identification of independent locus presumed to be controlling these various protein fractions. A summary of experimental details of electrophoresis of each enzyme system studied in the present investigatioon of M. cephalus is given in Figure 68-77 and Plate 22-24B, 26-29. A comparison and evaluation of these details of standardization clearly revealed lot of valuable informations. For instance, it was essential to compare each of the twelve protein systems in M. cephalus from each tissue so as to enable to discover genetic variations at different loci. Figure 4 (ACP), shows that TGH buffer system could reveal only very feable enzyme activity in serum and liver tissue whereas all other five different tissues showed no activity of the enzyme at all. Similarly, a comparison of results obtained using other buffer systems (FIG. 5, APC, TVB) for resolving ACP from these same tissues, exhibited sufficient enzyme activity in all the tissues. Thus the buffer system and the tissue showing as best source of the required enzyme pattern were selected as standardized parameters in terms of total number of clear bands, staining intensity (IX, 2X, 3X) of individual bands and nature of separation and resolution (P, G, B) of bands as shown in the Table 4 (ACP, BOR). The results of such experimental standardization from all fourteen protein systems produced many valuable informations as depicted in the form of self explanatory photographs, figures and tables (FIG. 2-57; PLT. 3-13; TBL. 4-17).

The results of these standardization procedures in the present investigation suggest that all known protein systems may be present in all the tissues of an organism and their presence can be made visible, if a suitable standardized electrophoretic method is formulated by trail and error. The other important informations produced by the present standardized methods are that the same identified enzyme expressed specific electrophoretic patterns in almost all tissues either in terms of total number of bands or relative

mobility (mm) of different bands or staining intensity of different bands or in combinations of any these factors (FIG. 2-57; PLT. 3-13; TBL. 4-17) etc. This is popularly known as tissue specific patterns of protein systems particularly in higher organisms. The occurrence of tissue specific protein patterns is a common phenomenon in vertebrate and invertebrate organisms (Tsuyuki et al., 1965a; Hongskul, 1968; Tsuyuki et al., 1968; Manohar and Velankar, 1973; Herzberg and Pasteure, 1975; Bhattacharya and Alfred, 1982; Salini and Shaklee, 1988). The present investigation also suggests that the visible degree of tissue specific variability of these enzymes may be due to fundamental differences in the physiological function of each of these tissues causing the observed tissue specific electrophoretic variability. It is interesting to observe that liver tissue was found as the best source of ten out of nineteen enzyme systems studied in the present investigation (TBL. 3). As liver is an important organ and plays a major role in various physiological functions of the organisms, all kinds of protein systems are expected to be naturally present in it. Therefore, liver may be considered as a better source of biochemical genetic variations. However, eye lens, muscle tissues and serum produced the best zymogram patterns for general proteins. These biochemical genetic characteristics detected by the standardized electrophoretic and histochemical staining techniques for the species are presented in the form of self explanatory photographs, figures and tables (FIG. 2-57; PLT. 3-13; TBL. 4-17). The biochemical genetic characteristics of M. cephalus studied here comprised of nine general protein loci and twelve enzyme loci of selected tissues (TBL. 21).

The results obtained clearly revealed the existence of high degree of variations in the biochemical genetic characteristics of the individuals of M. cephalus examined in the present investigation. Since the first discovery of three different human haemoglobin phenotype polymorphism and its demonstration by electrophoresis by Pauling et al. (1949), such variations, popularly known as biochemical genetic polymorphism, are found to be a common phenomenon in both invertebrate and vertebrate organisms. Using starch gel electrophoresis Smithies (1955) reported group variations in the serum proteins of normal human adults. The biochemical reason for the different electro-

phoretic behaviour of these haemoglobin phenotypes was later given by the British biochemist Ingram as differences in amino acid composition in the polypeptide chains by way of single amino acid substitution (Verma and Agarwal, 1978). Thus Ingram was able to modify the earlier one gene-one enzyme hypothesis put forward by Beadle and Tatum (1941) into one gene-one polypeptide hypothesis. It was also proven beyond doubt that all proteins including enzymes in all organisms, irrespective of their structural and functional differences, are under the control of genes coded for them. It is also known that the proteins composed of amino acids form the structural basis of all organisms.

Twenty naturally occuring amino acids, in random combinations, can produce an infinite order of protein types to create the biological diversity detectable and visible at cellular, tissue and individual levels of the organisation. Thus, proteins present in any organisms are an excellent source for biochemical genetic investigation. Hence, to study the biochemical genetics of M. cephalus it was essential to examine as many proteins as practically possible in the present investigation. Twelve protein systems present in M. cephalus could be examined during the investigation period (TBL. 23). The major reason for selecting these twelve protein systems was that biochemical genetic techniques to obtain their zymogram patterns were already available in the published reports (Shaw and Koen, 1968; Smith, 1968; Brewer, 1970). These twelve protein systems studied in M. cephalus were identified as two general protein systems and ten enzyme protein systems.

In the present investigation on the biochemical population genetics of M. cephalus these essential basic data have been collected from different zymograms obtained for the species. The term "Zymogram" was given by Hunter and Markert (1957) to describe the coloured enzyme patterns detected by histochemical staining techniques on starch gel after electrophoresis which was already introduced by Smithies (1955). Markert and Muller (1959) suggested the word "Isozyme" for enzyme patterns thus obtained by the above techniques. As some of these "Isozymes" like Lactate Dehydrogenase (LDH) were found to exist in multiple forms. Wroblewski and Gregory described them as

"Isoenzymes" (Latner and Skillen, 1968). As there appears to exist confusions in the use of these terms and as the objective of the present investigation was to study the biochemical genetics of M. cephalus, the common practical term enzyme systems was used to describe each enzymes studied here. Similarly, electrophoretic patterns of non-enzymatic and enzymatic proteins obtained are described as Zymogram patterns in the present investigation.

Since the discovery of high degree of enzyme polymorphism in natural populations of Drosophila (Lewontin and Hubby, 1966) and human populations (Harris, 1966), populations of different species including fishes have been found to show genetic polymorphism at a number enzymatic and non-enzymatic protein loci. Handy references on detection of different protein systems like "Chromatographic and Electrophoretic Techniques" (Smith, 1968) and "An Introduction to Isozyme Techniques" (Brewer, 1970) have enabled researchers to report polymorphism in a number of protein loci in both invertebrate and vertebrate species. Details of polymorphism detected and observed by these above mentioned techniques at 21 different loci in 12 different protein systems of sample populations of M. cephalus studied in the present investigation are presented in the form of zymograms (FIG. 68-77; PLT. 22-29) and other details as shown in Table 23.

In a population sample all the expected genotypes need not be present. Besides, the frequency of each genotype can also vary among population samples. And finally genotype frequencies depend on the present population genetic structure and sample population size (Strickberger, 1968; Ayala and Kiger, 1980). These basic aspects of genotype distribution at particular locus are also reported by other investigators (Smith et al., 1978; 1980; Basiao and Taniguchi, 1984; Fevolden and Haug, 1988). The present interpretation of observed phenotypic variations as genotype variation at 21 presumed loci can be conclusively proved only by breeding experiment which is neither practical nor possible under the present investigation. The artificial hybridation experiments of species of salmonidae showed that parental muscle protein patterns are inherited by the hybrids (Tsuyuki and Robert, 1963). Numerous

reports of species and tissues specific phenotypes of general proteins are indirect evidences of genetic nature of muscle protein detected by various electrophoretic methods (Connel, 1953; Tsuyuki and Robert, 1963, 1966; Tsuyuki et al., 1965b, 1966a, 1966b, 1968; Mackie, 1968; Mackie and Jones, 1969; Kasinathan et al., 1972; Manohar and Velankar, 1973; Natarajan et al., 1975; Taniguchi and Sakata, 1977; Bhattacharya and Alfred, 1982). However it does not mean that every observed general protein variation is genotype variation. Depending on developmental, physiological or environmental conditions, certain fractions of muscle protein in certain species may show non-genetic differences. It is upto the researchers to observe, identify and differentiate such non-genetic variations present from that of genetic variations. Another important prerequisite in this respect is correct identification of the species to be investigated from similar species occuring in the same area. Such species specific and ontogenetic differentiations made in the present investigations are discussed first followed by the results obtained from each protein system.

5.1 SPECIES SPECIFIC BIOCHEMICAL GENETIC CHARACTERISTICS OF M. CEPHALUS

Mullet species are morphologically almost similar and hence correct identification of different species is a practical problem (Thompson, 1966; Luther, 1975; Rao, 1981). Accurate identification of species is a prerequisite in any form of fisheries research, particularly, in genetics. Therefore, it was important to study the species specific biochemical genetic characteristics of commonly available mullet species from local waters where from a sample of M. cephalus was also collected for the present investigation. A comparison of zymogram patterns obtained from twelve protein systems was made to reveal species specific characteristics of M. cephalus, L. parsia and V. cunnesius commonly available in Cochin (FIG. 58A - 62C; PLT. 14A - 18B; TBL. 18). Though all these individual zymogram patterns showed species specific characteristics, the degree of variations and resemblances can be described in three different ways. First, a group of zymogram patterns which

show distinct patterns for each of the three species. The first group consisted of patterns of ACP, ADH, GPD and SDH. The visible differences are mainly in the form of either the total number of bands or their distinct electrophoretic positions or by both (FIG. 58A, B, 59D, 62B; PLT. 14A, B, 15D, 18B; TBL. 18). The second group consisted of species specific distinct zymogram patterns shown only between any two species. These patterns were ADO, ALD, AKP, EST, GDH, LDH (E), LDH (M), MDH, ODH, PRO (E), PRO (M) and PDH (FIG. 58C, D, 59A-C, 60A-D, 61A, B, 62A, C; PLT. 14C, D, 15A-C, 16A-D, 17A, B, 18A; TBL. 18). In the second group, the differences are also caused by either the number or electrophoretic position of the bands or by both. The results show more resemblances between L. parsia and V. cunnesius than between either of these and M. cephalus. Whether the L. parsia and V. cunnesius are genetically more closer than M. cephalus is a phylogenetic question to be investigated in terms of interspecies genetic distance. The third group is composed of those patterns showing much resemblances between the zymogram patterns of two species with sufficient minor variations still showing their species specific characteristics.

This is probably the first report of comparison of seventeen different protein systems consisting of fifteen enzymes and two general proteins in these three species. The species specific genetic informations made available in the present investigation should help in any future biochemical genetic research programmes in these three species. In this respect the preliminary informations recorded in the dissertations work of Mary (1985) and Prag (1984) are also to be considered. Mary (1985) observed species specific electrophoretic patterns of muscle proteins and seven enzymes in M. cephalus and L. parsia. Similarly Prag (1984) also observed tissue specific and species specific electrophoretic patterns in L. parsia. There are numerous reports of species specific biochemical genetic characteristics revealed by gel electrophoretic techniques. Tsuyuki et al. (1968) reported species specific protein differences in scorpaenid fishes. Tsuyuki et al. (1965b) reported species specificity and constancy of muscle and haemoglobin in fishes. Sulya et al. (1961) reported species specific plasma protein of fishes including M. cephalus and M. curema from the Gulf

of Maxico. Similarly Senkevich and Kulikova (1970) reported species specific serum protein patterns in M. cephalus, L. salines and L. aurata. Herzberg and Pasteur (1975) were able to provide species identification key for mullet species of the eastern Meditarenian waters based on differences in electrophoretic pattern of muscle protein. Rao (1981) reported species specific electrophoretic patterns of LDH in seven species of Mugilidae. He also reported species specific electrophoretic pattern of Alcohol Dehydrogenase, Esterase and Muscle protein in three species of mugilidae.

A comparison of electrophoretic patterns of esterase, alcohol dehydrogenase and general protein of muscle and eye lens reported in M. cephalus by Rao (1981) with that of the present investigation shows some differences. He reported one banded esterase in heart tissue whereas two banded esterase was observed in the present investigation. The possible cause for showing the pattern difference must be due to differences in the methodology adopted in these two investigations. In the case of alcohol dehydrogenase the patterns reported by Rao (1981) and the patterns observed in the present investigation are almost similar. In this case, the methodology followed is almost same. However, the patterns of muscle proteins reported in M. cephalus by Rao (1981) showed considerable difference in the number of bands compared to that of the present investigation, it being seven and fifteen respectively. The major reason for the difference must be due to different buffer systems used in these two cases, namely, Sodium dodecyl sulfate (SDS) gel electrophrosis in the former and Tris-Glycine-HCl Buffer system in the latter.

The electrophoretic patterns of LDH eye lens in M. cephalus as reported by Rao (1981) and observed in the present investigation is identical, it being five banded isoenzyme patterns. However Rao (1981) reported a single banded LDH in muscle whereas two bands were observed in the present investigation in M. cephalus. The difference in these two results may be due to size differences of the specimens used in these two experiments. Because, muscle LDH pattern in M. cephalus was observed as size dependent upto certain size in the present investigation. The specimen measuring and tested up to

10 cm as having a single band and in specimen measuring 28.5 cm and above two bands. All these above reports, particularly, the present report of species specific patterns of fifteen enzyme systems and two general protein systems prove, beyond doubt, that biochemical genetic characteristics can readily solve apparent taxanomic problems found especially in the family Mugilidae.

5.2 ONTOGENETIC VARIATION

In biochemical genetic investigations, zymogram patterns of different proteins and enzymes presumed to be under the control of independent locus are compared to collect basic data required for computation of genotype frequencies between populations. A possible error in scoring phenotype variations as genotype variations can occur, due to developmental or ontogenetic variations produced by differences in size, sex, maturity stages or even environmental factors. In the present investigation, precautions were taken to avoid scoring such phenotype variation possibly produced by the above mentioned factors. Only consistant patterns expected according to codominant allelic variations were scored and interpreted as genetic variations. As a precaution, the eye lens and muscle protein systems which contributed nine polymorphic loci out of seventeen polymeric loci studied in M. cephalus were compared in different size groups. The eye lens protein patterns are shown in Figure 66 and Plate 21A. The results showed that specimens measuring 2.6 to 3.1 cm (Group III) were lacking certain protein fractions expected at five loci regions as observed in larger specimens used for scoring genotype variation. However, size 9.5 to 10.0 cm (Group II) started showing expected patterns as lightly stained bands. The lightly stained bands of smaller size became well stained thick bands in size 28.5 cm and above (Group I). Thus the ontogenetic effects, if were any, started disappearing from about 10 cm size (FIG. 66; PLT. 21A). The specimens tested for biochemical genetic variation in the present study were all above 15 cm. Similar size associated variation in eye lens protein in M. cephalus from Hawaii with regard to a particular fraction was reported by Peterson and Shehadeh (1971). Such size dependent eye lens protein variations in M. cephalus was also suspected by Bhosle (1977).

Muscle general protein patterns in M. cephalus were also compared in different size groups as in the case of eye lens protein mentioned above. The results are shown in Figure 67 and Plate 21B. In specimens measuring 2.6 cm the bands appeared to be very lightly stained though all the expected fractions at four loci are visible to certain extent indicating lesser quantities of protein in each fraction. However in specimen measuring 3.1 cm upwards the patterns were almost same as expected showing increased staining of the fractions. However, in size above 10 cm, the last fifth fraction which was lightly stained in 2.6 cm but strongly stained in 3.1-9.5 cm group appeared to be stained very lightly again indicating much lesser quantity of protein. Similar observations were made by Herzberg and Pasteur (1975) in juvaniles of M. cephalus, and L. aurata. The fast moving fraction decreased in intensity with growth and disappeared entirely in adult.

Though the LDH pattern in eye lens in M. cephalus tested here was consistantly five banded in all larger specimens tested from Cochin, Madras and Orissa, in specimens measuring 2.6 to 3.1 cm only one lightly stained band was present occupying the position of first slow band of five banded pattern (FIG. 64; PLT. 20A). The LDH enzyme being a tetramer showed five isoenzyme fractions in hybrid molecule. The presence of a single fraction at the slowest band position in smaller specimen mentioned above appears to suggest the enzyme present in eye lens in M. cephalus may be comparable to LDH A subunit identified in higher vertebrates.

A comparison of muscle LDH pattern in M. cephalus measuring different sizes indicated some ontogenetic differences as shown in the Figure 65 and Plate 20B and it is distinctly different from that of eye lens. The LDH enzyme activity in specimen measuring 2.6 cm was represented by a single lightly stained band which in specimens measuring 3.1 cm showed increased staining activity indicating increased amount of enzyme. In specimens measuring 9.5 and 10.0 cm the band in the same position, as in the small specimen, showed more enzyme activity and also showing a lightly stained additional slower moving fraction. In specimen measuring 28.5 cm there were two bands instead of a single band at the same identical position where highly

stained single band was observed in small specimen. Thus there appeared to be some form of ontogenetic variation in LDH enzyme in eye lens and muscle tissues in M. cephalus until a particular stage of its development.

A comparison of phenotype patterns of Alkaline Phosphatase in three different size groups of M. cephalus showed variations that appeared to be size dependent (FIG. 63; PLT. 19). The group 15.5 to 17 cm showed enzyme activity at 15 to 25 mm gel area whereas the second group 15.5 to 19.5 cm showed enzyme activity at 10 to 15 mm whereas in larger size group 31 - 31.5 cm, the enzyme was active at 8 to 10 mm gel area only. Thus these phenotype variations indicated higher amount of Alkaline Phosphatase in smaller M. cephalus specimens. These ontogenetic informations gathered at the initial phase of the investigation enabled to avoid possible errors in the genetic interpretations presented here. The phenotype variations interpreted as genetic variations are discussed below at different headings.

5.3 GENERAL PROTEINS

5.3.1 EYE LENS PROTEIN (PRO (E))

The zymogram patterns of eye lens proteins in M. cephalus population samples from Cochin, Madras and Orissa are shown in the Figure 74 and Plate 27. Zymogram patterns of eye lens in each region showed phenotype variations at particular gel area. These phenotype variations known as polymorphisms were presumed as biochemical genetic variations produced by a particular protein locus designated as PRO (E). Such locus controlled eye lens proteins are common in fishes and have been reported in a number of other vertebrate organisms (Smith, 1962, 1966a, 1966b, 1969, 1971, 1990; Barrett and Williams, 1967; Smith and Goldstein, 1967; Eckroat and Wright, 1969; Peterson and Smith, 1969; Peterson and Shehadeh, 1971; Menezes, 1976a, 1976b; Rao and Dhulked, 1976; Bhosle, 1977; Smith and Jamieson, 1980.

A general comparison of observed phenotype variations in all the three regions enabled to identify five independent loci responsible for all protein bands observed in all the three regions (FIG. 74; PLT.27). A variation of this nature and order is also not a rare phenomenon. Eye lens proteins having

a monomeric structure and two alleles can produce three genotypes. A visual comparison of observed eye lens protein genotypes at five independent loci in M. cephalus populations from Cochin, Madras and Orissa revealed significant differences between these populations (FIG. 74; PLT. 27). Though the first eye lens protein locus, PRO (E)-I was monomorphic in Cochin and Orissa, it was polymorphic in Madras. Though the second locus of eye lens protein, PRO(E)-II was polymorphic in Madras and Orissa, it was monomorphic again in Cochin. Monomorphism at these two loci appears to be due to the fixed allele designated as slow **(S)** in the respective regions. On the other hand at the fourth eye lens protein locus, PRO (E)-IV, the fixed allele occured in Madras and Orissa was designated as fast **(F)**. The fourth locus was polymorphic only in Cochin population where both alleles were present. Interestingly, the third and fifth loci were polymorphic in all the three regions. Thus the observed presence or absence of polymorphism in one or more of the five loci of eye lens proteins in three different populations of M. cephalus suggests that they may be genetically different populations.

These genotype differences or resemblances were again reflected by the corresponding gene frequencies estimated for all the five loci (TBL. 21). Some striking gene frequency differences are noticeable between Cochin and Madras, Cochin and Orissa and between Madras and Orissa. For example when first two loci are monomorphic with cent percent **S** genes in Cochin there is only ninety per cent **S** genes in Madras. Similarly, fourth locus in Cochin was polymorphic with seventy five percent **F** allele whereas it was monomorphic with cent percent **F** allele in Madras and Orissa (TBL. 21). Again considerable gene frequency differences occured between Cochin and Orissa with reference to second, fourth and fifth locus. An additional difference between Cochin and Orissa is also reflected by the type of predominant allele present in these two regions at fifth locus. When **S** allele was predominant in Cochin it was **F** in Orissa. Though gene frequency differences exist between Madras and Orissa at loci-I, II, III and V it was of lesser degree than that between Cochin and Madras.

This is for the first time a detailed investigation on the biochemical genetic nature of eye lens general proteins of M. cephalus of India from three distant regions was carried out. The only known published report on eye lens protein variations in M. cephalus from India is that of Bhosle (1977). As his methodology was different as well as information reported being preliminary in nature, his data cannot be compared with that of the present detailed studies. Bhosle (1977) has just reported five different eye lens protein patterns based on total number of bands and described these as three, four and five banded phenotypes. His final conclusion was that these different patterns suggest heterogeneity or intraspecies polymorphism in M. cephalus. The few specimens tested appear to be from Goa region. Depending on the sample population, seven to ten variable phenotypes were observed in the present investigation (TBL. 23). Based on eye lens protein variation, Peterson and Shehadeh (1971) also reported five phenotypes in M. cephalus from Hawaii. He suspected sub-population differences in M. cephalus in Hawaiian waters. His conclusion was again based on mere differences of total number of eye lens protein bands detected on cellulose acetate paper as Bhosle (1977) also used for his investigation of M. cephalus from Goa water. These differences reported by these two authors were not analysed at gene loci level as genotypes and their frequencies. Nevertheless, these two reports indicated that eye lens protein in M. cephalus may be a potential biochemical genetic marker for identifying its different genetic stocks. It is interesting that Peterson and Shehadeh (1971) and Bhosle (1977) using same cellulose acetate medium got only five bands in two geographically distant populations. The cellulose acetate paper used by these authors being a ready made paper and resolves and separates only major bands the resemblances in their results are not surprising inspite of the populations tested are from two far away geographical regions. On comparisons, the eye lens protein variations in M. cephalus reported in the present investigation are more informative and superior. Because a total of thirteen genotypes out of fifteen expected were discovered in different populations of M. cephalus tested from three regions (TBL. 23) in the present investigation. These basic informations are of fundamental

importance and essentially required for biochemical genetic characterisation of the species from its different geographical regions. Hongskul (1968) reported genetic stock differences in M. cephalus populations from east and west coast of Australia. Serum protein showed genetic polymorphism at a particular locus in east coast whereas it appeared to be non-polymorphic in the western population. Similar genetic situations were observed in the present investigation in M. cephalus in different loci, namely, absence of polymorphisms at PRO (E) -I in Cochin and Orissa populations whereas polymorphism at the same locus in Madras population. Again, PRO (E) II was polymorphic in both Madras and Orissa populations, whereas it was non-polymorphic in Cochin. PRO (E) IV was polymorphic in Cochin whereas it was non-polymorphic in Madras and Orissa. Similar genetic differences observed in muscle protein loci in M. cephalus studied in the present investigation are discussed elsewhere.

The biochemical genetic characteristics of eye lens protein have been investigated and applied at inter and intra species levels of various species of fishes (Smith, 1962, 1965, 1966a, 1966b, 1969, 1970, 1971; Barrett and Williams, 1967; Smith and Goldstein, 1967; Eckroat and Wright, 1969; Peterson and Smith, 1969; Menezes, 1976a, 1976b; Smith and Jameison, 1980). Intra-species variations in eye lens protein reported in fishes like yellow tuna Thunnus albacares (Smith, 1965), Ocean White fish Caulolatilus princeps (Smith and Goldstein, 1967), brook trout Salvelinus fontinalis (Eckroat and Wright, 1969), sand bar shark Carcharhinus milberti (Peterson and Smith, 1969) and mackerel scad Decapterus pinnuatus (Smith, 1969b). On the other hand absence of intraspecies variations were also reported in many fishes like Atlantic mackerel (Smith, 1962; Barrett and Williams, 1967; Menezes, 1976a; Smith and Jamieson, 1980; Smith et al., 1981b). All these above reports on eye lens protein in fishes reveal its potential as a genetic tag in solving problems associated with different levels of species organisation.

5.3.2 MUSCLE PROTEIN (PRO (M))

The second protein system in M. cephalus that has been investigated in the present study is muscle protein or myogen. The muscle protein

zymograms obtained for individual population samples from Cochin, Madras and Orissa are shown in the Figure 75 and Plate 28. An overall comparison of all the muscle protein zymograms for these three regions revealed intra-species polymorphisms controlled by four presumed independent loci. The very nature of muscle protein genotypes and distributions of gene frequencies indicate strong genetic differences among three populations. The presences of high polymorphism at PRO (M) I with three genotypes **(S, SF** and **F)** in Madras and absence of polymorphisms at the same locus in Cochin and Orissa indicate strong genetic differences between these populations (TBL.23). Though the monomorphic allele in both Cochin and Orissa was the same **F** allele, these two populations have to be considered as genetically different. Because these two monomorphic populations are situated at two distant latitudinal regions with polymorphic Madras population in the middle latitude. Similar but more important evidence of strong genetic differences existing among these three populations are clearly indicated by muscle protein locus PRO (M) IV. Though, Madras population is polymorphic, Cochin and Orissa populations are monomorphic for the same locus. On the contrary the monomorphic allele in Cochin and Orissa are entirely different, these being **S** allele in Cochin and **F** allele in Orissa (TBL. 23). Another striking genetic difference that clearly separates Madras population from that of Cochin and Orissa is that all four muscle protein loci are highly polymorphic in Madras while only two are polymorphic in Cochin and Orissa. Thus significant difference observed in the distribution of various genotypes in four muscle protein loci of M. cephalus suggests that its populations in Cochin, Madras and Orissa are genetically different stocks.

It also appears that this is the first report on the discovery of muscle protein polymorphism in M. cephalus species and its application in the identification of its different genetic stocks. Intraspecies muscle protein variations have been reported in many other fish species like Chanos chanos (Winans, 1980), Lates calcarifer (Salini and Shaklee, 1988). The present finding of muscle protein variation in M. cephalus while corroborating similar phenomenon

in other species revealed its potential as a genetic marker in fish stock differentiation. Jamieson and Turner (1980) applied muscle protein genetic differences to distinguish anguilla species of American and European origin. Tsuyuski et al. (1965a, 1965b) reported species specificity and consistency of muscle protein in numerous fish species and its application in biochemical systematics of fishes.

5.4 ENZYMES

5.4.1 ACID PHOSPHATASE (ACP)

Acid Phosphatase second locus (ACP II) appeared to be a suitable genetic marker for differentiating genetic differences in M. cephalus populations examined in the present investigation (FIG. 68; PLT. 22). An overall comparison of different zymogram patterns of Acid Phosphatase second locus revealed the existence of five phenotypes and four alleles **(S, S1, F, F1).** Maximum number of four alleles observed among twenty one loci examined in M. cephalus is at ACP II locus. When all these four alleles were present in Orissa population only two in Cochin and three in Madras were observed. In these four alleles **S** and **S1** appear to be rare alleles. Though these two rare alleles were present in Orissa, only **S** allele was present in Madras and none in Cochin. In this regard, these three populations appear to be genetically different. The other two alleles **F** and **F1** though present in all the three populations, considerable frequency differences of a particular allele, in a particular population, also suggest that these three populations are genetically different. The predominant allele in Cochin (58%) and in Orissa (64%) was **F1** whereas the allele **F** was predominant in Madras (62%) (TBL. 21). Though the frequency of the predominant **F1** allele in Cochin and Orissa is nearly similar, the presence of another different predominant allele, namely, **F** in Madras keeps Cochin and Orissa populations, as already discussed in the case of muscle protein **(PRO (M))**, without mixing in genetic sense. Thus these three populations are isolated and genetically different.

Though several fish species have been investigated by others for detecting genetic variant forms of acid phosphatase, it has been reported as

monomorphic in Cichlids (Kornfield and Koehn, 1975), guppy (Shami and Beardmore, 1978), trout (Wishard et al., 1980), halibut (Fevolden and Haug, 1988) and perch (Seeb and Gunderson, 1988). On the other hand many crustaceans like penaeid spp. (Mulley and Latter, 1980), P. kerathurus and P. japonicus (De Matthaeis, et al., 1983), P. indicus and P. stylifera (Philip Samuel, 1987) rock lobster (Smith et al., 1980) and lobster (Tracey et al., 1975b) showed polymorphism at acid phosphatase locus. Philip Samuel (1987) found different gene frequencies for acid phosphatase between Cochin and Bombay populations of P. stylifera whereas its frequencies were similar in other populations of P. stylifera and that of P. indicus. These reports and findings of the present investigation suggest that acid phosphatase can become a potential biochemical genetic marker for stock differentiation of both fishes and shell fishes.

Philip Samuel (1987) reported two banded ACP heterozygote as having a monomeric enzyme structure. Similar patterns were observed in the present study of enzyme in M. cephalus. However, it is also known that acid phosphatase exhibit a dimeric enzyme structure having three banded heterozygote. Similar variations reported in other enzymes as observed in the present investigation have been discussed in the section aldolase.

5.4.2 ALDEHYDE OXIDASE (ADO)

Aldehyde Oxidase (ADO) was also found highly polymorphic in M.cephalus with two alleles (FIG. 69 and PLT. 23A). Its gene frequencies were same in both Cochin and Madras populations having a frequency of 0.63 and 0.37 in each population. However, it was considerably different in Orissa population having equal frequencies for both alleles (TBL. 21). Thus the gene frequency difference at ADO locus could indicate the genetic heterogeneity of Orissa population. The available reports on biochemical genetic studies in other fishes show that ADO was not investigated as a potential genetic marker. It has been studied in detail in Drosophila (Ayala et al., 1974). It has been found monomorphic in many marine crustaceans (Lester, 1979,

1983; Fuller and Lester, 1980; Redfield et al., 1980; De Matthaeis et al., 1983) as reported by Philip Samuel (1987). However, it was found polymorphic in P. indicus showing two alleles with two banded heterozygote showing monomeric structure for the enzyme (Philip Samuel, 1987) as also observed in M. cephalus studied here.

5.4.3 ALDOLASE (ALD)

Aldolase (ALD) enzyme in M. cephalus was found polymorphic in Cochin and Orissa and monomorphic in Madras. Lack of polymorphism at ALD locus in Madras and its highly polymorphic nature in Cochin and Orissa indicates significant genetic difference of M. cephalus population in Madras (FIG. 70; PLT. 23B). This finding strongly supports similar genetic difference indicated by esterase locus discussed elsewhere. Though both slow and fast aldolase alleles were present in Cochin and Orissa, considerable differences in the aldolase allele frequencies between Cochin and Orissa also suggest that these two populations may be genetically different stocks as also revealed by esterase locus discussed later. The predominant allele **F** had a frequency of 0.54 in Cochin whereas it was 0.76 in Orissa (TBL. 21). Besides, the middle geographical position of monomorphic Madras population should keep polymorphic populations of Cochin and Orissa as genetically isolated as also discussed at certain other loci.

Aldolase enzyme appears to have not been well investigated in other species of fishes. It was found monomorphic in lake trout (Dehring et al., 1981). Its distribution in different tissues of yellow tail (Seriola quinqueradiate), flat fish (Microstoma kitaharae), swell fish (Spheroids pardalis) and sea bream (Sebastodes matsubarae) was reported (Takeo, 1970). It was monomorphic in limulus (Selander et al., 1970) halibut (Fevolden and Haug, 1988). Its distribution in Tilapia zillii showed five banded in white muscle and kidney whereas brain showed only a single band (Cruz et al., 1982). Philip Samuel (1987) studied aldolase enzyme variations in marine prawn species. He reported monomeric three alleles in P. indicus. Other prawn species showing aldolase allele variations was P. japonicus (De Matthaeis et al., 1983). A comparison

of heterozygote structure of aldolase in the present investigation with that of Philip Samuel (1987), De Matthaeis et al. (1983) shows similar monomeric enzyme structure. However, aldolase (E.C. 1.2.13) was reported as tetrameric, mainly in mammals (Ward, 1978). However, it is not unusual to observe a different subunit structure in different species and in different tissues in the same species. Similarly malic enzyme a tetramer in mammals reported as monomer in fish (Frydenberg and Simonsen, 1973) and amphibian (Hedgecock and Ayala, 1974). Glucose-6-phosphate Dehydrogenase a dimer in mammals was found as tetramer in fish and amphibian (Yamauchi and Goldberg, 1973; Hori et al., 1975). Superoxide dimutase in man also exists as dimer and tetramer (Harris et al., 1977; Ward, 1977). All these reports as mentioned in Ward (1978) suggest that expression of chemical structure of enzyme or isoenzyme can vary from species to species and even between loci within the same individual.

5.4.4 ESTERASE (EST)

The zymogram patterns of esterase enzyme system in heart tissue of populations of M. cephalus tested from Cochin, Madras and Orissa showed genetic polymorphism at its single locus (FIG. 71; PLT. 24A). Though Cochin and Orissa populations showed two of the three expected genotypes, Madras population possessed its characteristic single homozygous genotype **(SS)**. The absence of fast homozygous genotype **(FF)** and heterozygous genotype **(SF)** in Madras populations thus suggests population of M. cephalus in Madras may be fixed for **SS** homozygous genotype and hence it may be genetically different from other two populations. The chances of discovering other two expected genotypes on further screening of larger population sample cannot be ruled out. However, as all the forty eight specimens tested from Madras being the same **SS** genotype, the Madras population may be naturally devoid of other two genotypes. This reasonable assumption is further strengthened by the fact that forty eight specimens from Cochin and Orissa showed all the three genotypes with their distribution in Hardy-Weinberg equilibrium condition. Besides, the above assumption of distinct genetic nature of Madras population is again strongly supported by other loci ALD and PRO (E) as

discussed earlier. Though M. cephalus populations of Cochin and Orissa showed two and three expected genotype respectively, that too, in Hardy-Weinberg equilibrium, these two populations possessed characteristic gene frequencies. The predominant allele in Cochin was slow **S** allele (83%) whereas it was fast **F** allele (87%) in Orissa (TBL. 21). These significant gene frequency differences indicate that populations of M. cephalus in Cochin and Orissa also are genetically different stocks. Thus the population characteristic fixed esterase allele in Madras and significant differences in the nature of predominant alleles in Cochin and Orissa indicate that M. cephalus populations in Cochin, Madras and Orissa are experiencing some form of reproductive isolation.

Altukhov (1981) detected similar reproductive isolation in populations of salmon (Oncorhynchus spp.) and red fish (Sebastes) based on esterase gene frequency differences. His testing the gene frequencies of these populations year after year also showed the same consistant degree of gene frequency differences indicating these populations are reproductively isolated. Avise and Smith (1974) detected significant gene frequency differences at esterase locus in blue gill Lepomis macrochirus as an evidence of genetic heterogeneity between reservoirs of the same drainage system. Stable genetic heterogeneity and reproductive isolation of local populations were detected by Altukhov (1981) in Pacific salmon and other salmon species by applying many enzyme gene frequencies including that of esterase. Krieg and Guyomard (1985) reported large genetic heterogeneity in brown trout (Salmo trutta) population based on many gene frequency differences including that of esterase. The nature of esterase showing two alleles designated as 100 and 105 in brown trout was identical to the type observed in M. cephalus. The 105 esterase allele was fixed in population No.19 while it was only the predominant allele (65%) in population No.20. Utter (1969) considered significant gene frequency differences calculated at esterase locus at a level of 0.603 and 0.828 between two populations of Pacific hake Merluccius productus as genetic stock differences. Significant gene frequency differences in esterase locus enabled Smith et al. (1978) to identify two distinct genetic stocks in New Zealand snapper Chrysophrys auratus.

Figure 71 and Plate 24A show esterase phenotype patterns observed in homozygous and heterozygous condition. Homozygous showed two bands and heterozygous showed three bands suggesting a dimeric enzyme structure for the heart esterase in M. cephalus. The esterase enzyme activity was detected using alpha napthyl acetate as substrate. Similar two banded homozygote and three banded as well as four banded heterozygotes were reported at EST locus 3 in the bluegill (Avise and Smith 1974). Usually alpha napthyl acetate esterase shows a monomeric enzyme structure. However, it was reported as dimeric in nature in mammals (Ward, 1978). Moreover, in certain species esterase enzyme appear to be monomorphic in particular locus in one tissue and dimeric in another locus in another tissue (Salini and Shaklee, 1988). Similarly, single banded and double banded homozygotes were reported at esterase loci I and II respectively in the same tissue (Avise and Smith 1974). Thus the subunit structure of esterase appears to vary even within the species and between the species. As the objective of the present investigation was to apply these enzyme patterns as genetic markers to differentiate the genetic stocks of the species studied, no attempt was made to characterise chemical structure of the esterase enzyme with reference to different enzyme substrate and other chemical factors.

5.4.5 ALPHA -GLYCEROPHOSPHATE DEHYDROGENASE (GPD)

Alpha-Glycerophosphate Dehydrogenase (GPD) was found highly polymorphic in nature in all the three populations of M. cephalus (FIG. 72; PLT. 24B). However allelic frequencies were sufficiently different to consider each population as genetically different. The predominant allele in Madras and Orissa was different, it being **F** allele (0.56) and **S** allele (0.62) in respective regions (TBL 21). The frequencies of **S** and **F** allele were equal in Cochin. Johnson et al. (1970) reported genetic variation of GPD in Pacific Ocean perch (Sebastodes alutus). The genotype variations showed a diallelic polymorphism with a dimeric structure of enzyme. They suggested its variations as potential genetic markers for differentiation of genetic stocks. The present findings of alpha-glycerophosphate dehydrogenase polymorphism in M. cephalus also agree well with a diallelic and dimeric structure of the enzyme. This

assumption is based on the single banded homozygote and three banded heterozygote. Wishard et al. (1980) studying biochemical genetic characteristics of native trout populations observed gene frequency differences in GPD enzyme in one of the hatchery population indicating its genetic heterogeneity. Similarly, Krieg and Guyomard (1985) also observed considerable gene frequency differences among French trout populations (Salmon trutta) revealing genetic heterogeneity. Based on significant gene frequencies in alpha-glycerophosphate dehydrogenase Busack et al. (1980) were able to differentiate genetically different Eagle Lake trout populations from other trout populations of other regions. Some populations showed two allelic polymorphism while others were found fixed for a particular allele.

Allendorf and Phelps (1981) was able to differentiate rainbow trout and cutthroat trout population using differences in frequencies of a particular allele of GPD. Smith et al. (1981b) reported almost similar low level gene frequencies of GPD in nine regional populations of New Zealand hoki Macruronus novaezelandiae. Guyomard (1981) and Thompson (1985) reported considerable gene frequency differences in GPD between different trout strains. Basiao and Taniguchi (1984) showed allelic differences in GPD between two Japanese stocks of tilapias. Two species were found fixed for different alleles. Taniguchi et al. (1983) were able to differentiate wild and hatchery populations of black sea bream by observing allelic frequency differences of GPD. It was polymorphic in wild population but monomorphic in artificially bred populations. McAndrew and Majumdar (1983) found GPD allele frequency differences as potential markers to identify different closely allied stocks of tilapia species. Koljonen (1986) found considerable differences in the GPD allelic frequencies of different rainbow trout strains of Finland. Sumantadinata and Taniguchi (1990) observed significant allelic frequencies differences in GPD between carp stocks of Indonesia and Japan. Thus GPD enzyme appears to be more polymorphic in nature than many other enzymes considered in all these reports including the present report on M. cephalus. It was also found polymorphic even in the so called phylogentic relic like the horseshoe crab, limulus (Selander et al., 1970). The genetic nature of GPD was also

substantiated by the report of its inheritance in the genetic cross experiment in the pink salmon by Aspinwall (1973).

5.4.6 OCTANOL DEHYDROGENASE (ODH)

Though Octanol Dehydrogenase (ODH) enzyme was found to be polymorphic in M. cephalus populations all the expected three genotypes were found only in Orissa population giving an allelic frequencies of 0.57 and 0.43 for **F** and **S** alleles respectively. Cochin and Madras populations showed only three banded heterozygote phenotypes thus showing equal frequencies for both alleles in these two regions (0.50) (TBL. 21). The presence of all the three phenotypes with different allelic frequencies in Orissa and absence of homozygous phenotypes in Cochin and Madras indicate that M. cephalus in Orissa is genetically different from that of Cochin and Madras (FIG. 73; PLT. 26). Reports on the application of ODH enzyme for genetic differentiation of fish populations are not many. Stoneking et al. (1981) reported significant ODH gene frequency differences between northern and southern populations of brook trout Salvelinus fontinalis. The ODH enzyme was also found polymorphic at very low level in two species of shark. Its gene frequencies were not different between the populations of each shark species (Lavery and Shaklee, 1989). It was found monomorphic in guppy (Shami and Beardmore, 1978) and halibut (Fevolden and Haug, 1988). It was found polymorphic, in P. indicus with three banded heterozygote (Philip Samuel, 1987). The enzyme was also found polymorphic in many other penaeid prawns (Mulley and Latter, 1980; Richardson, 1982b) as reported by Philip Samuel (1987). The presence of single banded homozygous and three banded heterozygous phenotypes in the present study of M. cephalus also shows that ODH is a dimeric enzyme.

5.4.7 PYRROLINE DEHYDROGENASE (PDH)

The pattern of allelic frequencies of Pyrroline Dehydrogenase (PDH) in three populations of M. cephalus tested from Cochin, Madras and Orissa strongly suggests that these three populations are genetically different. The phenotype scored as heterozygote was having more than double the enzyme

activity of homozygous type. Thus homozygous was narrow banded and heterozygous broad banded. The population from Madras showed only the presumed heterozygous phenotype whereas Cochin and Orissa showed both homozygous and heterozygous phenotypes (FIG. 76; PLT. 29). Thus the allelic frequencies in Madras was equal whereas it was unequal namely 0.83 for **S** allele and 0.17 for **F** allele in Cochin and almost similar frequencies in Orissa (TBL. 21). The Madras population with considerable gene frequency differences occupies latitudinally almost the middle position with respect to Cochin and Orissa population. It naturally suggests geographical and genetic isolation of these three populations. The same natural situation was present at general protein locus PRO (M) III discussed elsewhere. Reports on the genetic variability of PDH enzyme are also not many. Probably this is the first report on its zymogram patterns from a fish. Philip Samuel (1987) reported monomorphic PDH in P. indicus and P. stylifera. It was found not very active in larval stages of P. esculentus and P. semisulcatus (Lavery and Staples, 1990).

5.4.8 TETRAZOLIUM OXIDASE (TZO)

The Tetrazolium Oxidase (TZO) enzyme activity reported in the present study of M. cephalus was observed along with aldolase enzyme phenotypes in liver tissue. As gel area of TZO enzyme activities were different from that of aldolase, scoring its phenotypes was not difficult. The TZO enzyme activities could be recognised not as coloured band but as colourless bleached area. As shown in the Figure 77 the TZO phenotype moved faster than aldolase enzyme. TZO enzyme activity was also observed along with many other enzyme tested in the present study like ADH, ADO, ODH etc. The TZO enzyme is also known as indophenol oxidase or superoxide dimutase (Hopkinson et al., 1976). However due to some unknown reason the TZO enzyme was not shown in most of the specimens from Orissa. Hence, the comparison of its polymorphic variation could be made only between Cochin and Madras. The enzyme showed two phenotypes namely, homozygous slow phenotype **S** in the form of a small band and heterozygote phenotype **SF** in the form of a large band. Here, again, as in the case of PDH, the difference between

homozygote and heterozygote was in the size of the band, the homozygote having a single dose and the heterozygote having a double dose enzyme activity. The genotype frequencies of the enzyme were identical in both Cochin and Madras populations. From the width of the homozygote and heterozygote enzyme activity the TZO enzyme structure appears to be dimeric in nature as reported in many other fish species where the heterozygote was reported as three banded (Shami and Beardmore, 1978; Smith et al., 1980; Guyomard, 1981). However it was also reported as two banded heterozygote in marine prawns (Mulley and Latter, 1980; Philip Samuel 1987) and four banded in European hake (Mangaly and Jamieson, 1978). Though TZO is dimeric, it can vary in its zymogram expression of heterozygote structure as also observed in the case of esterase enzyme discussed earlier. The reason for the apparent absence of TZO in M. cephalus from Orissa may be associated with experimental causes like weak activity of the enzyme itself and, therefore, was not visible enough for easy detection. Another reason may be that the active area of the enzyme was masked by the staining background. Yet another possible reason may be that the genetic stock structure difference of Orissa population might have affected its detection. A repeated testing of a large sample from Orissa alone will reveal the real cause of its absence in the present investigation.

5.4.9 GENERAL ASPECTS

In biochemical genetic investigation, it is also important to report whether distribution of observed genotypes at each locus in each population is according to the expected genotype distribution estimated as per the Hardy-Weinberg Law of genotype distribution (Ayala and Kiger, 1980; Utter, 1987). A comparison of the observed and expected genotype distributions in seventeen gene loci tested in M. cephalus showed significant deviation with P values between just less than $P < 0.01$ and more than $P > 0.001$ with different degrees of freedom at about nine loci (TBL. 23). These significant deviations are caused mainly by excess of heterozygotes in nineteen places out of twenty four compared for the purpose (TBL. 25). As it is well known that Hardy-

Weinberg equilibrium condition between observed and expected genotype frequencies can occur only in an ideal population structure or an ideal statistical situation which is not affected by the factors such as mutation, migration, genetic drift and selection etc. The occurrence of excess of homozygotes or heterozygotes or otherwise their deficiencies in certain loci is not a rare phenomenon in species of fishes, crustaceans and molluscs as reported by many investigators. It is interesting to note that in most of these reports deviation from H.W. equilibrium condition is due to excess of homozygotes or deficiency of heterozygotes. For example, excess of homozygotes reported in anchovies, stolephorus spp. and spratelloides spp. (Daly and Richardson, 1980), Chanos chanos (Winans, 1980), mackerel (Smith et al., 1981a) marine molluscs (Singh and Green, 1984; Zouros and Foltz, 1984), marine prawns P. indicus and P. stylifera (Philip Samuel, 1987) and sharks (Lavery and Shaklee, 1989).

A few reports of significant deviation from H.W. equilibrium condition due to excess of heterozygotes are also available as observed in the present investigation. Report of excess of heterozygotes in eye lens protein in locus in M. cephalus population from Hawaii (Peterson and Shehadeh, 1971) is strikingly interesting and important. Similar excess of heterozygotes was also observed in eye lens protein in M. cephalus populations from Cochin, Madras and Orissa as reported in the present investigation. These identical situation of excess of heterozygotes in M. cephalus tested by two different investigators using two different electrophoretic methods suggest that the observed excess of heterozygotes must be due to similar natural phenomenon existing in two far away geographical locations and not due to experimental errors or artifacts. In this respect, it is also logical to infer that observation of excess of heterozygotes in all other loci in M. cephalus tested in the present investigation are real. The other reports of excess of heterozygotes are in lake white fish Coregonus clupeaformis (Imhof et al., 1980), lake trout Salvelinus namaycush (Dehring et al., 1981) and Atlantic salmon Salmo salar (Stahl, 1987; Verspoor, 1988). The possible reasons given for the occurrence of excess of heterozygotes

in populations of different species above are, as usual, presumptive rather than experimental proofs. Excess of heterozygotes can occur due to small number of parents during the breeding process or possible statistical type I errors (Stahl, 1987), duplication of locus (Stoneking et al., 1981) as referred by Dehring et al. (1981), effect of larger year class spawned when there is more interbreeding between populations (Imhof et al., 1980). Two reasons given above (Stahl, 1987 and Verspoor, 1988) are mainly with reference to hatchery stocks of salmon and hence may be due to the effect of hatchery procedures. The possible reason for the occurrence of excess of heterozygotes in the present investigation of M. cephalus may be due to small number of parents available during breeding process. Because it appears that M. cephalus fishery is dwindling in numbers during the last few decades as informed by the local fishermen engaged in capture of mullets using Chinese dipnets.

As nineteen out of twenty four significant deviations observed in the present investigation were due to excess of heterozygotes it may be suggested that some form of heterozygous advantage exist in M. cephalus causing the observed unequilibrium condition in genotype distribution. The heterozygote advantage in organisms sometimes is described as the phenomenon of heterosis, particularly, produced by two different strains (Strickberger, 1968). A comparable "hybrid vigour" produced even in ordinary cases of heterozygote formation can lead to excess of heterozygotes as observed in the present case.

In the present investigation, excess of homozygotes in three enzyme loci were also observed, particularly, in M. cephalus population from Cochin area whereas it occurred in only one enzyme locus in Madras (ACP II) and Orissa (ADO). It may suggest some unknown special situation favouring excess of homozygotes at three loci in Cochin population (ACP II, ADO; GPD; TBL. 25). Though different possible reasons for excess of homozygotes in different reports are available such as Wahlund effect (Hedgecock, 1977), due to mixing of genetically distinct populations (Smith and Jamieson, 1978), selection pressure of various nature (Smith and Jamieson, 1980) and null allele (Grant et al., 1987), these cannot be logically considered as reasons for the

present observation of excess of homozygotes in M. cephalus. Because out of twenty four significant deviation observed in the present investigation only five were caused by excess of homozygotes. As excess of heterozygotes are overwhelming in M. cephalus the occurrence of few cases of excess of homozygotes in the same species could be due to some other unknown factors controlled by ecological or other factors in Cochin population. Smith et al. (1978) also observed excess of heterozygotes in esterase (EST 4) and excess of homozygotes in GPI I in New Zealand snapper Chrysophrys auratus. The reason for observation of excess of heterozygotes as well as homozygotes in different loci in New Zealand snapper as also observed in M. cephalus of the present investigation may be mixing of genetically different stocks. As out of five occasions of excess of homozygotes in M. cephalus three were from Cochin alone, the possibility of mixing of locally differentiated stocks may be possible as suggested in the case of New Zealand snapper. This can be tested by comparing large sample populations from different areas around Cochin. Another possible reason may be that these three enzyme loci which cause excess of homozygotes are weak particularly with reference to any one allele in activity. Thus wrong scoring of the phenotype could have caused counting more homozygotes. It is also possible that some unknown mechanism of phenotype selection exist that favours homozygotes in certain loci while favouring heterozygotes in other loci in the same individual. Such a mechanism may also be under the pressure of local environmental conditions. Apparent correlations between excess of heterozygotes in fishes and different environmental conditions have been reviewed by Ponniah (1989). A comparison of reports reviewed there reveals the complexity of the probable mechanism involved in the observed excess of heterozygosity at loci like esterase, lactate dehydrogenase, transferin, phosphoglucomutase and glycerophosphate dehydrogenase in different fish species. A typical example given in the review is excess of heterozygosity by the abyssal population of the fish Sebastodes alutus at PGM and GDPH loci while lack of excess heterozygosity in shallow water population as reported by Johnson et al. (1970). Now the relevant question to be raised with regard to the observation of excess heterozygosity in M. cephalus is that whether the excess of heterozygotes in its populations

at Cochin, Madras and Orissa is due to similar selection pressure existing at all the loci in all these three regions. Until some confirmative experimental evidences are collected, it is not advisable to suggest a mechanism for the excess heterozygosity in M. cephalus.

In biochemical population genetics, gene frequencies are of fundamental importance to get an insight into the genetic nature of the natural populations throughout its range of distribution. The degree of species evolution through space and time can be evaluated by comparing gene frequencies of different populations. Because, differentiation of populations towards different levels of speciations occurs by changes in the frequencies of the genes and genotypes (Dobzhansky, 1967, 1970; Ayala, 1975). The important question now arises is that whether the significant gene frequency differences observed and reported in the present investigation at fourteen out of twenty one loci discussed above can really indicate that M. cephalus populations from Cochin, Madras and Orissa are experiencing some form of barriers like ecological, geographical or even reproductive isolation itself so as to create the type of gene frequency differences observed in these three populations (TBL. 21). As it is well known that gene frequency can really be different in two or more populations if they have been isolated for long period of time by any one of the above barriers (Altukhov, 1981). Signs of such isolation of populations can be detected by testing and conforming gene frequency differences on repeated periods of intervels as shown in the case of trout populations (Altukhov, 1981). It is also known that gene frequency is a function that may be affected by the population size from which the sample was drawn and also by the sample size itself.

The next question to arise is whether the sample size has affected the results. In a diallelic locus, three genotypic individuals can exist and hence all these three genotypes can turn up at a specific point of random sampling. Considering the statistical impracticality of obtaining all these three genotypes in a sample of three, it is a common practice to obtain a sample as large as possible. Though sample size larger than 10 individuals

are satisfactory in biochemical genetic studies, Utter et al. (1987) suggested a sample size of 50 to 100 for obtaining statistically meaningful data. In this respect, the present sample size of about 50 is reasonable and satisfactory. The choice of small sample size in the present experiment was influenced by the constraints of very high cost of the specimens, its availability and time limit of the investigation etc. On the other hand the pattern of similar gene frequencies shown existing in some of the loci like ODH, PRO (E) III, PRO (M) II in all the three populations and between Cochin and Madras etc. as shown in table rules out of the cause of present observation of gene frequency differences in three populations of M. cephalus as due to small sample size. Inspite of small sample size when one or more particular loci are monomorphic in one population and polymorphic in another, genetic differences are strongly indicated (Utter, 1987; Jamieson 1974b). In this respect, the present observation of absence or presence of polymorphism at certain loci (TBL. 21) as already described, strongly support that these three populations are isolated in some respect and are genetically different stocks.

The information on frequencies of heterozygotes, polymorphic loci and average number of alleles in each population is no less important in biochemical genetic characterisation of individual populations than the gene frequencies themselves as discussed above. These estimates can bring out genetic variability of the species as well as probable differences of genetic variability between populations (Ayala and Kiger, 1980; Ryman and Utter, 1987). Therefore, it is a standard modern practice to quantify these three frequencies and to report the degree of genetic variability of the species investigated. The high degree of genetic variability in M. cephalus studied here is clearly evident in the average proportions of heterozygotes observed at 21 loci (TBL. 24) and number of alleles (TBL. 22). For example, overall mean heterozygosity for M. cephalus is 0.36 (TBL. 24) which is comparatively much higher than reported in other species of fishes. The average heterozygosity for 14 species of fishes with 21 loci for species was only 0.08 (Ayala and Kiger, 1980). Thus the present observation of 0.36 heterozygosity in M. cephalus thus becomes a typical pattern of genetic variability as reported

in European hake (Mangaly and Jamieson, 1978) where the value was 0.33. However, the number of loci examined by Mangaly and Jamieson (1978) were only limited and hence the genetic variability estimate may be on the higher side than it is in the species. Genetic variability at 0.09-0.10 levels was reported in Atlantic cod, Gadus morhua (Grant, 1987) and in guppy Poecilia reticulata (Shami and Beardmore, 1978) respectively. The present estimate of 0.36 average heterozygosity appears to be the highest ever reported in any group of organisms. Its estimate in flatfish Pleuronectes platissa showed 0.17 (Ward and Galleguillose, 1977). It may be meaningless to try to assume the reasons for the apparent unusual higher genetic variability recorded in M. cephalus. It becomes higher only when compared to heterozygosity estimates made for other organisms. On the other hand, the value 0.17 is much close to the heterozygosity estimate 0.15 for drosophila and land snails (Ayala and Kiger, 1980). Expecting values of experimental research of one person to be comparable to that of others cannot be a true scientific approach. When the inter locus average genetic variability in M. cephalus is significantly different from its own overall average in the species, it is no wonder genetic variability cannot be expected to be of statistically similar in all species of fishes. It may depend on the species, the loci tested and a number of other still unknown factors. For example, inter locus heterozygosity in M. cephalus tested in the present case varied from 0.00 to 1.00 at Cochin and Madras populations whereas it was 0.00 to 0.75 in Orissa. Similarly interpopulation heterozygosity in M. cephalus varied from 0.00 to 0.92 at ALD locus between Cochin, Madras and Orissa populations and it was almost similar at PRO (E), PRO (M) and TZO loci. These statistical averages can become meaningless in certain situations when one higher value compromises with another lower value so as to produce the so called overall average for the species as explained above and noticeable as recorded in Table 24. Such statistical artefacts naturally reflected in every reports of genetic variability can produce, on comparison, apparent higher or lower values than expected. This is evident in the Table 18:11 of Ayala and Kiger (1980) where average heterozygosity for each group under invertebrates, vertebrates and plants and their overall averages are given. The groupwise average heterozygosity

value for vertebrate varied from 0.042 to 0.082 showing almost cent precent increase in the value whereas for invertebrates its range was from 0.062 to 0.151 showing an increase of one and half times in the value. Thus average heterozygosity value of 0.17 reported in flatfish by Ward and Gallequillose (1977) also becomes a typical compared to 0.078 for fourteen fish species referred by Ayala and Kiger (1980), the former being more than double of the latter. In this respect, the present report of 0.36 average heterozygosity is more than a double of 0.17 reported by Ward and Galleguillose (1977). The higher heterozygosity value of 0.36 in M. cephalus may also be correlated to much higher average frequency value of polymorphic loci of 0.67 in the species (TBL. 20). This is again much higher than 0.306 for fourteen fish species (Ayala and Kiger, 1980; TBL. 18: 11). A question yet to answer is what would have been the average heterozygosity value if more than 21 loci, say, 50 or so, were examined in M. cephalus. As per Ayala and Kiger (1980) an electrophoretic survey of about 20 loci is usually sufficient and screening of 71 loci has not changed the heterozygosity value earlier obtained by 26 loci in humans. However, as genetic variability and its mechanism may vary from species to species, a survey of larger number of loci in M. cephalus alone can further confirm the atypical heterozygosity in M. cephalus.

This is very well reflected in the various ways and means researchers sought in explaining the levels of heterozygosity in their experimental animal as Smith and Jamieson (1980) particularly referring to the probable reasons for reporting a comparatively high heterozygosity of 0.11 by Somero and Soule (1974) whereas about four times lower value of 0.03 in the same species by Siebenellar (1978). The probable reasons Smith and Jamieson (1980) trying to point out were again products of comparisons of values of different nature such as non-enzymatic and enzymatic protein based heterozygosity estimates or glucose and non-glucose metabolising or even internal and external substrates, single and multiple substrates and lastly the monomeric and multimeric structure of the enzyme itself. The final discussion of Smith and Jamieson (1980) about values on the hypothetical conditions for low and high genetic

variability shows that these results are again found contradicting between species. Hence, the best reasonable explanation for the observed apparent atypical 0.36 heterozygosity value in M. cephalus is that either it may be natural for M. cephalus or the mechanism of genetic variability and its variations between loci, populations, regions, species and higher levels of species organisation is too complex at the present level of our understanding.

Table 20 also shows a very high average frequency of polymorphic loci (0.67) in M. cephalus, a value much higher than 0.306 reported for 14 fishes by Ayala and Kiger (1980). Similarly, a survey of levels of genetic variation in 12 marine species including M. cephalus from different thermal regions by Somero and Soule (1974) showed an average frequency value of only 0.28. In the same report the average value reported for M. cephalus collected from Mission Bay and San Dugo (Pacific) was 0.37 which is considerably higher than the average for the 12 species compared. It is also interesting that Coryphaenoides acrolepis belonging to Macruridae family of fishes showed an average frequency of 0.667 polymorphism out of six loci tested (Somero and Soule, 1974). This is very closely comparable with the frequency value 0.67 reported in the present investigation in M. cephalus (TBL. 20). Thus the range of frequency of polymorphic loci detected by electrophoretic techniques can be very high, it being as low as 0.00 - 0.01 to the highest 0.67 in M. cephalus and as reported in C. acrolepis. Therefore, expecting a standard average frequency of polymorphic loci in a particular fish species is not advisable. The reason for high range of genetic variability between species and between regions cannot be justifiably correlated to any comparable phenomenon as it is an individual observation without any preconcept. Because when an average 26 loci in 44 prawn species were surveyed the percentage of polymorphic loci varied from zero in Hippa pacifica, Matuta planipes, Pachygrapsus transversus to 41 in Emerita analoga, Callinectes arcuatus (Nelson and Hedgecock, 1980; TBL. 2). This species dependent genetic variability was again reflected in heterozygosity values compared in the above report, it being 0.4 in Pachygrapsus transversus to 12.8 percent in Callinectes arcuatus (Nelson and Hedgecock, 1980; TBL. 2).

In a codominant allelic polymorphic system, a minimum of two variant alleles are observed at each locus. However, average number of alleles per locus tested can be less than one depending on the number of polymorphic loci present in the total number of loci tested. Thus average number of alleles can also be an indicator of genetic variability of the species and their populations. In the present investigation, the average number of alleles in M. cephalus was 1.77 even when 21 loci were considered. The values between Cochin, Madras and Orissa were closely comparable, these being 1.72, 1.82 and 1.78 respectively (TBL. 22). As the best index of genetic variability is heterozygosity itself (Ayala and Kiger, 1980; Utter, 1987), a detailed discussion on the average number of alleles is not attempted here. Moreover, average number of alleles in M. cephalus is naturally expected to be high as the values of proportions of polymorphic loci and heterozygosity were higher than usual. Average heterozygosity and percentage of polymorphic loci in M. cephalus have already been discussed earlier.

5.5 GENETIC IDENTITY AND DISTANCE

In modern biochemical population genetics, heterogeneity or homogeneity of two or more populations indicated by values of allelic or gene frequencies is further measured and reported in terms of genetic identity (I) and distance (D) as proposed by Nei (1972, 1975). Genetic identity (I) or genetic similarity is an estimated average measure of the proportions of structurally identical gene (alleles) detected at many loci between two populations. The (I) value can vary from 0 to 1, the former indicates lack of genetic identity, that is, no common alleles are shared, while the latter value indicates the two populations are identical, that is, the common alleles occur at identical frequencies in both populations compared. Genetic distance (D), on the other hand, is a measure of identity (I) in terms of "the number of allelic substitutions per locus in the separate evolution of two populations" (Ayala and Kiger, 1980). Thus the values of genetic distance may be described as an indirect index of evolutionary distance moved away by the concerned populations. In this respect, the genetic distance value (D) can range from zero to greater

than one. Thus the two ranges can correspond to no allelic substitution ever taken place or it has taken place more than once as evolutionary differentiation goes on between the two populations (Ayala and Kiger, 1980). A comparison of these values (I and D) in different groups of organisms including fishes in Table 22.3 of Ayala and Kiger (1980) suggests that a particular standard range of these values cannot be fixed for measuring the degrees of genetic similarity or heterogeneity of two populations of different species in their course of evolutions. Because genetic distance values required to recognize two samples, as just local populations in different species, can range from 0.013 in Drosophila to 0.020 in fishes, 0.053 in reptiles to 0.058 in mammals. The D values required to differentiate them as sub-species are again can range from 0.163 (Drosophila and Fishes), 0.232 (Mammals) and to 0.306 (Reptiles). Thus, when genetic distance values reach a range of 0.16 to 0.30 between two populations of many species including fishes they are to be viewed as attaining the genetic divergence level of sub-species (Ayala and Kiger, 1980).

The genetic identity and distance values computed for three populations of M. cephalus in the present investigation are much greater than the apparent standard values discussed above for local populations, these being 0.891 (I) and 0.157(D) respectively (TBL. 26). The D values given in table show a range of 0.095 to 0.233 between populations suggesting their divergence into a level of genetic distance lying between races and sub-species. The average distance shown for M. cephalus species was 0.157. Among three populations compared, namely Cochin, Madras and Orissa, greater genetic distance is shown between Madras and Orissa populations which are situated on the same east coast rather than between Cochin and Madras which are situated on the opposite east and west coast.

Though such unusual genetic distance values are very rarely reported at interpopulation levels, closely comparable atypical range of genetic distance values of 0.092 to 0.204, with an average 0.152 were reported in mosquito fish populations (Hernandez-Martich and Smith, 1990). The latest report of

most unusual range of genetic distance existing between Atlantic herring populations is 0.005 - 0.010 to 0.888 - 1.086 (Jorstad et al., 1991). The D values 0.888 to 1.086 were for two adjacent populations. The average D value 0.152, between populations of mosquito fish Gambusio holbrooki is very close to average D value 0.157 obtained in M. cephalus populations reported here. Such high D value is often expected to exist between species level differentiation (Thorpe, 1982) as reported by Hernandez-Martich and Smith (1990). Genetic drift is popularly considered as a basic reason for such high level genetic differentiation. Considering other known population characteristics of the mosquito fish, Hernandez-Martich and Smith (1990) suggested locality specific selection strategy for the observed unusually higher values for heterozygosity, allelic frequency and genetic distance. It is also interesting to note that Spatial genetic differentiation occured within a drainage rather than between two drainages except one particular drainage population which showed higher genetic differentiation. They suspected "interaction of biogeographic factors, gene flow, selection and stochastic factors determine the genetic characteristics of local populations of G. holbrooki.

On the other hand values of genetic distance in Chanos chanos populations were found to be too small to consider them as different populations though they originated from 10,000 kilometers range of their distribution. The average genetic distance was only 0.002. Interestingly, two populations separated by 320 km only showed significant genetic divergence due to their isolation by local oceanic conditions created (Winans, 1980). Hence, isolation by some form of physical barrier between two populations rather than long distance also appears to be an important cause more genetic differentiation of fish stocks as demonstrated by the above example. Another concrete example of the effect of barrier in genetic differentiation is the significant range of genetic distance values, viz. 0.13 - 0.36 reported for shore fishes separated by Isthmus of Panama though some of which looked morphologically alike (Gorman et al., 1976; Gorman and Kim, 1977; Vawter et al., 1980) as explained by Rosenblatt and Waples (1986).

In a genetic comparison survey of populations of several shore fish species isolated by oceanic distances, it was reported that their genetic distance values are surprisingly low, the range for different species tested being only 0.01 to 0.06. Two populations of M. cephalus from eastern Pacific and Hawaii regions, inspite of their isolation by long distance, showed a low genetic distance of 0.03 (Rosenblatt and Waples, 1986). The major reason for such low value of (D) inspite of transpacific distance, as the authors themselves suspect, is due to very small sample size, it being only a total of six specimens. Hence, heterozygosity was not estimated as reported. The other reason is the low level polymorphism detected in M. cephalus, it being only 4 out of 36 loci (Rosenblatt and Waples, 1986). Considering the unusually small sample size, its results cannot be compared with that of the present investigation. The only clear comparison is the detection of 4 loci in muscle protein in both investigations.

The average value of genetic distance (D) obtained for M. cephalus populations in the present investigation was 0.157 which is much higher than that reported by Rosenblatt and Waples, (1986) for trans Pacific M. cephalus populations as discussed above. The higher level of polymorphism and heterozygosity detected in M. cephalus of the present investigation suggests that the genetic strategy of Pacific and Indian M. cephalus may be highly different and, hence, the values of genetic distance in these two populations can also be of different order. This reasoning appears to hold good in the light of recent report of lack of genetic differentiation among nine populations of M. cephalus collected from, a distance of about 2000 km from east and west coast of Florida Peninsula. However, the only lacuna that stands against a reasonable comparison with that of the present result is that the enzyme systems tested by them are different from that of the present investigation (Campton and Mahmoudi, 1991). The above report suggests that the taxonomic status of the species known as M. cephalus through out the world may be different and hence a world wide biochemical genetic comparison of the species is essential.

In population characteristics, such as species distribution and migratory behaviour during breeding and spawning periods etc. the euryhaline species, like M. cephalus, Lates calcarifer and Chanos chanos are comparable. It is interesting to know that out of eight populations of L. calcarifer tested from different rivers of Northern Australia, seven were genetically discrete stocks (Shaklee and Salini, 1985; Salini and Shaklee, 1988). Though genetic distance values were not computed in their studies, other basic genetic differences in terms of gene frequencies, polymorphic loci, heterozygosity etc. were sufficient to reveal the heterogeneity of the stocks.

In the light of the above discussion regarding the probable causes of two populations of a species of wide distribution differentiating into genetically discrete stocks, it is natural to search for explanations for observing atypical values of genetic distance among M. cephalus populations tested from Cochin, Madras and Orissa. Geographically the above three populations can be considered as two different groups, populations of Cochin being from West Coast (Arabian Sea) and the other two being from East Coast (Bay of Bengal) of India. These two groups are separated by the Southern region of the Indian Peninsula. The only course of their mixing is by way of migration of adults or distribution of eggs from West Coast to East Coast or vice versa. The Peninsular geographical barrier existing between the East and West coast can really prevent such course of mixing, unless adults migrate towards a single spawning ground as is well known in the case of Atlantic eel (Jamieson and Turner, 1980). The available informations on the spawning activity of M. cephalus of the above three regions do not support such an unusual spawning behaviour. These reports confirm quite independent regional spawning behaviour. The peak spawning season for M. cephalus in Pulicat Lake, Madras was October to December (Rangaswamy, 1972b) September to December in Chilka Lake (Jhingran and Natarajan, 1969) September - December in Kayamkulam and Vembanad Lakes (John, 1955; Luther, 1973; Kurian, 1975). These authors also report that these spawners make seaward migration during the spawning season. However, further details such as how far they migrate and whether they have a common breeding ground etc. are not studied or

known. The observation of specimens with ripe and running ova conditions and collection of eggs and larvae from respective regions immediately after spawning suggests they are not having a common breeding ground. The actual range of breeding migration of M. cephalus populations in these three regions can be known only by well planned tagging and recovery programme. Such studies conducted elsewhere showed that tagged M. cephalus was caught from the same area months after the release while some were caught at a considerable distance. Some breeders return to the river they left, possibly indicating spawning was close by (Thompson, 1966). Again, there are contradicting conclusions made after such tagging and electrophoretic studies in M. cephalus (Campton and Mahmoudi, 1991).

A strong basis for suggesting that M. cephalus populations from Cochin, Madras and Orissa are genetically different stocks is the occurrence of region-wise fixed loci in the present investigation. These are EST in Madras, PRO (E) I in Cochin and Orissa, PRO (E) II in Cochin, PRO (E) IV in Madras and Orissa and PRO (M) I and IV in Cochin and Orissa. The fixed alleles at PRO (M) IV being different between Cochin and Orissa have become the stronger evidence of their being genetically isolated (TBL. 21). Thus mixing of these populations for possible gene flow may be ruled out. Rosenblatt and Waples (1986) suggested that the presence of two fixed electrophoretic differences is roughly equivalent to a D value of 0.06 indicating lack of gene flow. In their studies one fixed difference was found between populations of M. cephalus from Hawaii and E. Pacific. Thus the significant differences in genetic identity (I) and genetic distance (D) values detected between M. cephalus populations from Cochin, Madras and Orissa must be a reflection of some form of their reproductive isolation and certain amount of genetic divergence.

6. CONCLUSIONS

The following important conclusions are drawn from the results of the present investigation on the biochemical genetics of M. cephalus.

1. The biochemical genetic variability of M. cephalus populations tested from Cochin, Madras and Orissa is higher than that of many other species of fishes. The above conclusion is mainly based on atypical value of 0.67 polymorphic loci and 0.36 heterozygosity estimated for the species (TBL. 20, 24).

2. Significant differences shown in the allele frequencies at majority of 21 loci tested in populations from Cochin, Madras and Orissa suggest that these populations of M. cephalus are genetically different stocks (TBL. 21).

3. The range of genetic identity (I) values of 0.859 to 0.921 and distance (D) values of 0.947 to 0.2325 suggests that populations of M. cephalus from Cochin, Madras and Orissa are undergoing certain amount of genetic divergence (TBL. 26).

4. These detailed biochemical genetic informations gathered for the first time for M. cephalus of India can become a strong basis for further similar investigation for the purpose of fisheries management and modern aquaculture practices of the species.

7. RECOMMENDATIONS

1. A detailed investigation on the biochemical genetics of M. cephalus populations from east and west coast of India using larger number of specimens in each sample is urgent to confirm the genetic stock discretness of the populations tested in the present investigation as well as other local and distant populations not tested so far.

2. As populations of M. cephalus of Cochin, Madras and Orissa appeared to be genetically very different, appropriate management regulations are to be formulated for conserving their genetic resources and also to augment their production.

3. A world-wide standard biochemical genetic comparison of populations of the species known as M. cephalus is essential to discover and measure the genetic status of different geographical regions.

8. SUMMARY

1. The thesis deals with the results of an investigation on the "BIOCHEMICAL GENETICS OF MUGIL CEPHALUS" from Cochin, Madras and Orissa. It is presented under the following major headings: Introduction, Review of Literature, Materials and Methods, Results, Discussions, Conclusions, Recommendations, Summary and References.

2. The introduction gives a brief account of historical and modern back ground on the stock concept in fisheries research and management, followed by the importance and potential role of biochemical genetics in the identification of natural units of fisheries management. It also explains the necessity of investigating the biochemical genetics of M. cephalus.

3. In the review of literature published reports relevant to biochemical genetics with special reference to that of general proteins and enzyme systems of fish populations were considered.

4. A detailed account of the source of experimental specimens, mode of collection, transportation, sample extraction, gel preparation/gel electrophoresis, buffer systems, staining procedures of proteins/enzymes, standardization of experiments, interpretation of electrophoretic data using basic formulae etc. are given in the materials and methods section.

5. Separate data collected on:

    1. standardization of experiments,
    2. species specific identity of M. cephalus,
    3. its ontogenetic variations,
    4. comparative zymogram patterns of 2 general proteins and 8 enzyme systems from Cochin, Madras and Orissa populations of M. cephalus,
    5. allele frequencies,

6. average polymorphic loci,

7. average number of alleles,

8. average heterozygosity,

9. observed and expected genotype frequencies present at 17 polymorphic loci from Cochin, Madras and Orissa populations and

10. average genetic identity (I) and genetic distance (D) present at 20 loci from Cochin, Madras and Orissa populations of M. cephalus etc. are explained in the section results using seventy seven figures, thirty one plates and twenty six tables.

6. During the course of discussions, the following original important findings were projected as a basis for the present objective conclusion on the biochemical genetics of M. cephalus.

1. For the first time species specific biochemical genetic identity of M. cephalus, L. parsia and V. cunnesius has been clearly established applying zymogram patterns of fifteen enzyme systems and two general protein systems.

2. Precautions were taken to avoid possible size dependent ontogenetic variations while estimating data on genetic variations.

3. This is for the first time the biochemical genetics of populations of M. cephalus from Cochin, Madras and Orissa were studied, analysed and reported. Analysis of 21 loci in selected tissues of M. cephalus showed 17 polymorphic and 4 non-polymorphic loci (TBL. 21). A detailed analysis revealed that there exists a very high degree of biochemical genetic variability in the species as expressed by average values of 0.67 polymorphic loci, 1.77 alleles per locus and 0.36 heterozygosity (TBL. 20, 22, 24).

4. Allele frequencies for each of 21 loci were estimated from the observed genotype frequencies of sample populations from

Cochin, Madras and Orissa. Allele frequencies at 14 out of 21 loci were significantly different among three populations. These differences were reflected either in the form of frequency of the same allele or different alleles or fixation of either of the alleles in one or other population (TBL. 21). These significant differences at allelic frequencies of several loci suggest these three populations are genetically different.

5. Expected genotype distributions according to Hardy-Weinberg Law deviated significantly at nine out of 17 loci. These deviations were caused mainly by the occurrence of excess of heterozygotes. Excess of homozygotes also occurred in three loci at Cochin. Possible reasons for excess of heterozygotes/homozygotes have been discussed.

6. The range of genetic identity and distance values computed between populations of M. cephalus from Cochin, Madras and Orissa were 0.859 to 0.921 (I) and 0.095 to 0.233 (D) respectively. The averages of these values for the species were 0.891 (I) and 0.157 (D) respectively. These values suggest that populations of M. cephalus from Cochin, Madras and Orissa are experiencing some form of reproductive isolation and undergo certain genetic divergence (TBL. 26).

7. Four important conclusions were drawn on the basis of the results of the present investigation.

8. Three recommendations were also made on the basis of evaluation of the results.

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